VOLUME 5 JANUARY, 1935 NUMBER 1

American Journal of Clinical Pathology

OFFICIAL PUBLICATION
THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

CONTENTS

PUBLISHED BI-MONTHLY BY THE WILLIAMS & WILKINS COMPANY
MOUNT ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.
Made in United States of America

Entered as second-class matter, January 7, 1931, at the Post Office at Baltimore, Md., under the act of March 3, 1879

THE PATHOGENESIS OF PRIMARY CANCER OF THE LUNG*

HENRY C. SWEANY

From the Research Laboratories of the City of Chicago, Municipal Tuberculosis Sanitarium, Chicago, Illinois

The inception and progress of pathological changes throughout the course of primary cancer of the lung, offers, perhaps, the best field for study of some of the unknown aspects of cancer. This is particularly true of the alteration in morphology and character of the cells, considered as indirect metaplasia in the Ribbert sense, or those undergoing a "histological accommodation" or anaplasia, as expressed by Hansemann.

It is not necessary here to attempt an historical discussion of the subject, nor to mention, except briefly, the intriguing problem of incidence which has brought the subject out of obscurity into every day prominence within a few decades. The increase has apparently been more than ten-fold in the last thirty years, although this increase is more apparent than real. Part of it has been due to a change in the pathological viewpoint. Formerly it was considered that an organ susceptible to metastatic cancer was not so prone to develop primary cancer. As a result, many primary cancers were considered metastatic, and prior to a correct understanding of the rôle of the basal cell layer of mucosa, many of the small round cell cancers were, no doubt, confused with sarcomas. With the discrepancy in pathological interpretation that existed at the end of the last century, it is also easy to account for confusion in clinical interpretation. Much of the increase is also directly accountable to increased longevity as a result of which there are practically double the number reaching the age for cancer of the lung as did fifty years ago. Another

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

important factor is the improvements in diagnostic methods. Negative bacteriological findings and positive roentgenological and bronchoscopic findings in chronic pulmonary affectations take many cases out of other groups and ultimately place them as cancers of the lung.

Other less important factors are the improvements in transportation, the tendency of people with obscure diseases to go to large clinics, increased zeal on the part of physicians in dealing with obscure diseases, and a general improvement in the enlightenment of the public regarding such matters.

Irrespective of these many valid reasons for explaining the increase, there is still much to be learned regarding the rôle played by certain irritants in causing the disease.

Dust, smoke, automobile gas, tar on the roads, et cetera, have been suspected, but after a careful analysis none of them is found to cause any more disease than exists without them, and there is a proportionate number that do not develop the disease at all.

There are a few other conditions, however, that seem to be related more closely to the cause of the disease. In this group may be listed tuberculosis, syphilis, chronic lung diseases, pneumonia, and particularly influenza. Askanasy[2] in Europe, and Winternitz[18] in America, amongst others, have shown how influenza destroys the mucosa of the bronchi, and that during regeneration all the characteristic phases of early cancer are simulated. The regeneration of epithelium may not be according to the original type cell, but many cell types may appear during the course of regeneration. The question is raised whether such cell changes may not be the precursors of cancer. The greatest objection to this theory is that the increase in cancer of the lung had begun before the influenza epidemic of 1918 and that Hueper[7] found no increase after the 1890 epidemic. Studies in other pulmonary affections have yielded like results. McKenzie,[10] Kitamura[8] and Friedländer[5] have described the regeneration of epithelium in acute and chronic diseases of the lung. Sweany, Stadnichenko and Henrichsen (9) have reported squamous and cuboidal cell regeneration of ulcers in chronic Friedländer's pneumonia. Similar regeneration is also observed in chronic

tuberculosis. In fact, Ewing considers that this disease is one of the chief causes of cancer of the lung.

Just as in the healing of other lesions, it is quite frequently observed that tuberculous ulcers heal and regenerate epithelium of a wide variety of types. Figures 1 to 4 show various types of regeneration from chronic fibro-ulcerative tuberculosis. Some of these cells resemble ciliated columnar, others squamous cells, and others cuboidal or columnar cells having squamous cell character-

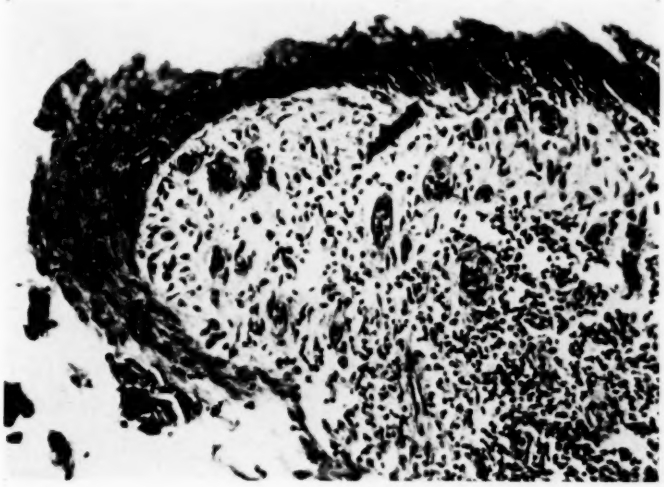

FIG. 1. OVERHANGING MARGIN OF SQUAMOUS EPITHELIAL CELL GROWTH OF A TUBERCULOUS CAVITY. × 188. HEMATOXYLIN-EOSIN

istics. There is a definite metaplasia of some of these cells to types not existing in a normal body. But in spite of this fact and the fact that many other healing tuberculous lesions have been studied where the epithelium has been regenerated, none has been found to be cancerous, indicating that the coincidence of cancer in such regenerating tissue is absent more often than present, and that the cause of malignancy must be sought elsewhere.

In a group of seventeen primary cancers of the lung, I have seen five with tubercle bacilli in the sputum. An interesting

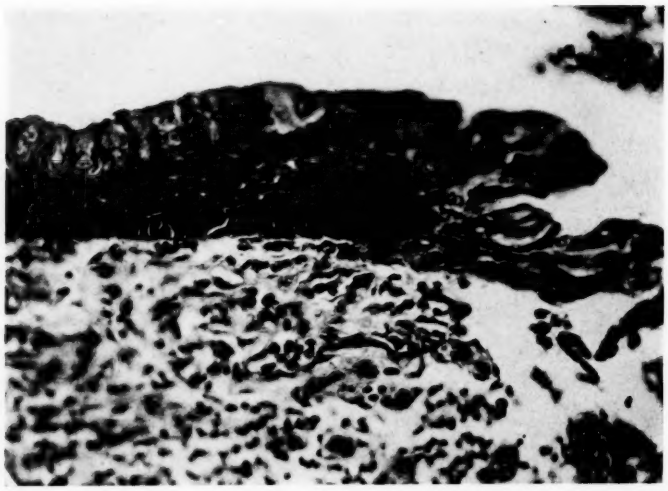

FIG. 2. A SIMILAR GROWTH TO THAT SHOWN IN FIG. 1. SHOWING A MIXTURE OF CUBOIDAL AND SQUAMOUS CELLS. × 188

FIG. 3. DIFFERENT TYPES OF COLUMNAR CELLS, SOME WITH CILIA. × 300

fact was observed, however, that so far as could be ascertained, not one of the cancers developed upon a tuberculous ulcer, but began on unaffected bronchi away from the active disease. In practically all, tuberculosis existed in the upper half, while the cancers were along the main bronchi or towards the base. The impression was gained from study that there was little or no relation between the two diseases.

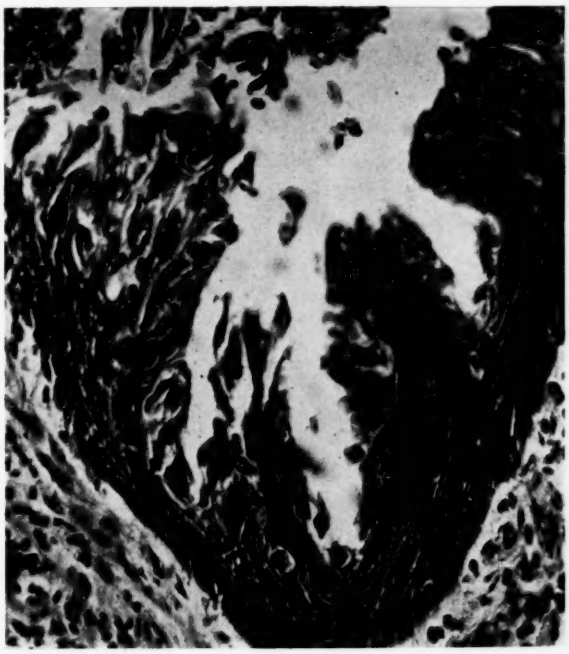

FIG. 4. COLUMNAR CELLS. HAVING SOME SQUAMOUS CELL CHARACTER IN THAT THEY TEND TO SHOW HYALINIZATION. × 300

Another objection to tuberculosis being a major cause of cancer of the lung is that the incidence of the two diseases in the last fifty years has been reciprocal.

Of other irritants, tobacco smoke has frequently been mentioned. Recently McNally[11] has pointed out the close parallelism between the increase in cancers of the lungs, and the consumption of cigarettes. In spite of the fact that this theory may

seem plausible, and that cancers have been found in inveterate smokers, there have been vast numbers of such smokers who have never developed cancer. If there is any merit to this theory it should soon begin to show in women who are now smoking almost as much as men, but who account for only about one-third the cancers of the lung.

Radium ore is a definite agent, however, that can be considered as a cause of cancer of the lung if one is to judge by the reports of Schmorl,[16] Arnstein[1] and Perchan and Sikl[14] in a study of the miners of this ore in Saxony and Bohemia. Realizing the effect of radium on other cells, such a result is to be expected. In this instance there seems to be a brutal change in the character of the germ plasm that leads to the anarchistic growth.

Such deep seated changes in the germ plasm seems to lie at the bottom of most cancers, whether they are produced abruptly by a substance like radium, or develop more slowly. There seems to be some inherent basic cancer tendency within the germ plasm before cancer will result. Radium seems to expedite or create this derangement. In people without this tendency, the many lesions described before will heal by a regeneration of epithelium, but will not become malignant until the inherent tendency within the germ plasm directs the growth into abnormal channels. When the basic germ plasm is sufficiently deranged, the regenerating epithelium will veer over into a malignant state. The immediate irritant may be an acute or chronic disease, tobacco smoke, or one of many others.

This theory will explain most of the cancers of the lung and will also assign an appropriate rôle for the various exciting or irritating factors that seem to be the actual cause in so many instances. Such a theory is expressed in substance by Fischer-Wasels.[3]

The earlier writers on the subject considered that the resemblance of the cancer cells to the various cells of the bronchial tract suggested an origin from the cell types they most resembled. Langhans, Chiari, and others, described the mucous gland types and ascribed their origin to the mucous gland cells; Ehrich and Paessler to bronchial epithelium; and Perls and Fuchs con-

sidered that squamous cell types came from alveolar epithelium. Later, it has been shown that the squamous cell types are not alveolar. Grünwald and Kretchmer report that there are perhaps very few, if any, true alveolar epithelial cancers. In view of these facts, and considering that in many cancers there are two or many types of cells in the same tumor, including squamous, ciliated, columnar, mucous gland cells, et cetera, one is compelled to pause before ascribing an origin from any one cell layer or type. It seems that the majority, if not all but an insignificant minority, arise from the bronchial mucosa in the light of present knowledge.

In order to understand existing conditions better, a brief review of the development of the bronchial epithelium will be helpful. As is well known, the origin of bronchial mucosa is from the inner surface of the lung bud, a purely epithelial structure. As with most epithelium, there are at least two layers of cells; one layer of adult cells to function, and another layer of basal cells to replace worn out or destroyed cells in the upper layer. In the bronchial mucosa there is the outer layer of ciliated columnar and mucous cells, a layer of intermediate cells, and the basal germinative layer, which are undifferentiated and possess embryonic characteristics.

As the lung develops, the layers of epithelium spread out over the smaller bronchioles and alveoli, gradually flattening out to a single layer of cuboidal cells, then flat plates over the alveoli. Cilia are not found in bronchioles smaller than 0.75 mm.

It is an open question yet whether the alveolar epithelium may not originate from mesodermal cells in adult life, as Fried,[4] Rose,[15] and others apparently believe. These authors have reported that phagocytic alveolar type cells have been seen to come from the deeper tissue of the alveolar walls in no way related to the superficial epithelium. Others, including Miller,[12] are of the opinion that it is an epithelial process throughout life. According to this view, regeneration must take place from higher up the bronchioles or bronchi, as exists in the regeneration of the mucous membranes. It will apparently require more research to settle the controversy.

As to the origin of cancers of the bronchi, the earlier work of MacCarty[9] on epithelial cancers in general, Moise,[13] Fried, Fischer-Wasels, and others, seems to be bringing order out of chaos and helping to clarify the problem of histogenesis. The bronchial mucosa is looked upon as a vital membrane that is equipped with an apparatus for removing irritating and noxious agents by means of the cilia and mucus. When this layer is injured the basal or germinative cells replace the destroyed cells of the upper layers. It is thought that this layer is of a multipotential nature, so that any type of cell along the bronchus may be reproduced as the necessity demands. Even the mucous glands seem to have originated from this basal layer and may be regenerated from it. As a result of this inherent nature, the basal layer may produce a mucous gland type of cell that gives an impression of a mucous gland origin of certain cancers of the lung. While it cannot be denied that certain cancers may come from mucous glands, it seems probable that the greater number, if not all, arise from the multipotential basal layer.

As the basal cells grow out, they may remain small and undifferentiated, resembling sarcomas; they may be larger and more typical basal cells, elongated to the "oat cell" type; they may be non-hyalinized squamous cells, hyalinized squamous cells, ciliated or non-ciliated columnar cells, mucous bearing cells, and a host of bizarre shapes and sizes that bear no resemblance to any body cell at all. The form taken by these various cells may be due to the character of the germ plasm when they are split off, with the possibility of the Ribbert type of metaplasia and the Hansemann anaplasia or histological accommodation altering them as the environment changes.

After once embarking on a malignant course, the speed of growth is quite variable. This is similar to other malignant conditions. There is no general rule, however, for predicting whether the tumor will be the rapid growing, infiltrating type, or the slow growing, circumscribed type. Generally the small basal cell types are rapidly proliferating and, therefore, run a rapid course. The less differentiated cells seem to be more embryonic and have a greater degree of vitality. As the cells be-

come more squamous, there is a tendency to be more circumscribed. As a result, the non-hyalinized cells become aged in situ and undergo hyalinization, presenting sometimes bizarre shapes and variegated staining characteristics. Perhaps age and other factors alter the individual cases so that there can be no definite course predicted from the cellular appearance.

The speed with which infiltration occurs does have a definite bearing on the distant metastases. Hruby and Sweany[6] pointed out that such growths penetrate the pulmonary veins early and cause early metastases by a direct scattering into the major circulation. As a result, metastases may occur in any part of the body, appearing most commonly in the lungs, liver, kidney, spleen, brain, cord, adrenals, et cetera. Metastases to the pleura and the hilum lymph nodes are encountered most frequently but are by direct extension and lymphatic spread, respectively. Other metastases are chiefly blood borune. It may be readily seen that the slowly proliferating types are the only ones that offer any opportunity for surgical aid.

As the tumors extend into the bronchi, there is an occlusion of the lumen that causes changes in breath sounds, dyspnoea, and other physical findings, as well as dilatation and formation of cysts and abscesses in the bronchi distal to the tumors. Growth into the pleural cavity leads to a rapid spread of the tumor around the pleural surface, encasing the lung in a tumor mass. This dense mass in the pleura causes the appearance of effusion on the roentgen ray and excruciating pain clinically. As the tumor mass becomes large, there is an impairment of circulation in the central portions of the mass, so that a resulting necrosis and carcinomatous abscess is sometimes the result. This sloughing is usually attended with hemorrhage, frequently fatal. This type of hemorrhage differs from the early infiltration of the mucosa where the newly formed capillaries exude hemoglobin into the mucus giving the "current jelly" sputum of Stokes.

After the tumor enlarges there is a resulting toxicity that leads to the graver symptoms of anorexia, loss of weight, anaemia, et cetera.

Finally, the development of metastatic tumor nodules may

cause a disturbance in any organ they chance to involve. They may locate in the central nervous system and spinal cord and cause symptoms that simulate almost any form of nervous system derangement. Metastatic growths in the other organs are not unlike those found in other forms of cancer.

Any attempt to connect the gross morphology of cancers of the lung with the histopathology, clinical or roentgen-ray findings, has met with failure. There is little in common except what has already been suggested. In this respect it is like cancers elsewhere. Where there is a prolific cell growth with many capillaries and but few fibroblasts, it may be termed medullary and a predominance of fibrosis may give the picture of a scirrous type. There may be all stages in between, some of which may resemble a carcinoma simplex.

Equally unsatisfactory is a classification according to other methods. Certain cancers are adenomatous, others are alveolar in morphology, others encepholoid, while a greater number are mixed. The alveolar types are interesting in that they are prone to develop along other connective tissue septa such as alveolar walls and lymph spaces. This tendency is sometimes so marked that there is only a replacement of the alveolar cells of the alveoli, thus simulating normal lung tissue. This is particularly true when the cells are of a low cuboidal type.

SUMMARY

The origin and pathogenesis of primary cancer of the lung has been discussed. With the exception of radio active dust, there are few, if any, tangible factors that can be accused of causing cancer of the lung. Various other diseases and irritants, however, seem to precede the onset of the disease in such a manner that a relationship is suggested. They perhaps cause a change in the layers of the mucous membrane, following which a regeneration occurs that is sometimes thought to become malignant. The trend of opinion at present is to view most of these factors as immediate irritants, with the true cause lying deeper in the germ plasm of the host. With a "cancer tendency" present, the basal layer of bronchial epithelium sometimes produces ma-

lignant growths in its attempt to replenish the injured layers of overlying mucosa. In this respect, it is not unlike malignancy of other epithelial structures. Most lung cancers are thought to arise from the basal layer rather than from mucous glands or pulmonary alveoli, if indeed they ever arise from these structures. After the growth begins, it may proliferate rapidly and occlude bronchi with symptoms of occlusion. It may also enter the pulmonary veins with a resulting distant and widespread metastasis. It may grow into the pleural cavity and encase the lung. A small number of growths may remain circumscribed for a long time before metastasizing, in which event there is the one and only opportunity for successful surgery of the lung.

REFERENCES

(1) ARNSTEIN, A.: Über den sogenannten "Schneeberger Lungenkrebs." Wien. klin. Wchnschr., **26:** 748–752. 1913.

(2) ASKANAZY, M.: Über die Veränderungen der grossen Luftwege, besonders ihre Epithel Metaplasie bei der Influenza. Cor.-Blatt f. Schweiz. Aerzte, **49:** 465–474. 1919.

(3) FISCHER-WASELS, B.: The origin of cancer. Jour. Am. Med. Assn., **102:** 214–215. 1934.

(4) FRIED, B. M.: Primary carcinoma of the lung: bronchiogenic cancer. Baltimore: Wm. Wilkins & Co., 1932, pp. 247.

(5) FRIEDLÄNDER, C.: Cancroid in einer Lungen Caverne. Fortschr. d. Med., **3:** 307–310. 1885.

(6) HRUBY, A. J. AND SWEANY, H. C.: Primary carcinoma of the lung with special reference to incidence, early diagnosis and treatment. Arch. Int. Med., **52:** 497–540. 1933.

(7) HUEPER, W.: Primary gelatinous cylindrical cell carcinoma of the lung. Am. Jour. Path., **2:** 81–90. 1926.

(8) KITAMURA, S.: Über sekundäre Veränderungen der Bronchien und einige Bemerkungen über die Frage der Metaplasie. Arch. f. path. Anat., **190:** 163–176. 1907.

(9) MACCARTY, W. C.: The histogenesis of cancer of the stomach. Am. Jour. Med. Sci., **149:** 469–476. 1915.

(10) MCKENZIE, I.: Epithelmetaplasie bei Bronchopneumonie. Arch. f. path. Anat., **190:** 350–367. 1907.

(11) MCNALLY, W. D.: Tar in cigarette smoke and its possible effects. Am. Jour. Cancer, **6:** 1502–1514. 1932.

(12) MILLER, W. S.: The epithelium of the lower respiratory tract. In Cowdry, E. V., Special Cytology, **1:** 71–87. 1928.

(13) Moise, T. S.: Primary carcinoma of the lungs. Arch. Int. Med., **28:** 733–772. 1921.

(14) Pirchan, A. and Sikl, H.: Cancer of the lung in miners of Jáchymov (Joachimstal). Am. Jour. Cancer, **16:** 681–722. 1932.

(15) Rose, S. B.: The finer structure of the lung. Arch. Path., **6:** 36–47. 1928.

(16) Schmorl, G.: Über den Schneeberger Lungenkrebs. Verhandl. d. deutsch. path. Gesellsch., **19:** 192–196. 1923.

(17) Sweany, H. C., Stadnichenko, A., and Henrichsen, K. J.: Multiple pulmonary abscesses simulating tuberculosis. Arch. Int. Med., **47:** 565–582. 1931.

(18) Winternitz, M. C., Wason, I. M., and McNamara, F. P.: The pathology of influenza. New Haven: Yale University Press, 1920, pp. 61.

THE DETERMINATION OF LEAD IN EXCRETA AND TISSUES*

ROBERT A. KEHOE

From the Kettering Laboratory of Applied Physiology, University of Cincinnati, Cincinnati, Ohio

The value of lead determinations on the excreta or tissues in relation to the diagnosis of lead poisoning is conditioned by the following considerations: (*a*) the analytical method employed must have a high degree of quantitative accuracy within certain well defined limits; (*b*) the samples of material to be analyzed must be collected with such care as to prevent contamination with lead; (*c*) they must be of such amount as to yield quantities of lead which are within the range of optimum sensitivity of the analytical procedures; (*d*) an adequate background of information must have been provided for the specific method used to enable the differentiation of normal and pathological findings. Failure in meeting any one of these requirements results in entirely worthless and misleading results, and since the difficulties will be apparent only to those who have had wide experience, each point will require discussion.

PREPARATION OF MATERIALS FOR ANALYSIS

All methods necessitate the removal of the organic material from the sample. Most workers agree that wet digestion with lead-free nitric, sulphuric, hydrochloric or perchloric acid, or various combinations of these depending upon the material to be analyzed, yields the best result. Some materials such as feces may be ashed in a muffle furnace at carefully controlled tempertures, after preliminary drying, but this method is not generally satisfactory in our experience. Methods for preparing various

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

types of materials have been detailed in the descriptions of methods to which I shall refer later.

ANALYTICAL METHODS

Various satisfactory methods have been described in the literature in step-by-step detail. I shall discuss only the several principles involved and cite the literature for reference. Each method has its advantages and its drawbacks, which it will be profitable to consider.

1. Quantitative separation of lead from other metals through its insoluble salts

Quantitative chemical separation of lead from other metals is accomplished most satisfactorily by the precipitation under suitable conditions of, first the sulphide, then the sulphate, and finally the chromate. Purely chemical methods which do not go through the sulphate step are unreliable, in that other metals may be carried along with the sulphides and precipitated with lead chromate. It must be stressed that unless interfering metals which may be present are completely eliminated, the analytical results are worthless. The final measurement of the lead as chromate has been made in various ways of which the most satisfactory, in my experience, is based on the color reaction of lead with pure S-diphenyl carbazide.[7] I have not found it possible to obtain the latter compound in sufficient purity and have been compelled to repurify it for use, but if this is done excellent results can be obtained with it. Others have used the thiosulphate iodometric titration of the chromate. It is inadequate, however, in dealing with the minute amounts of lead that are often found in the urine.

Various workers, conspicuously Avery and his associates,[1] Taylor,[12] and Tannahill,[11] have used purely chemical methods successfully. They depend for their final determination on the colorimetric estimation of lead as the sulphide, or as the acid sulphite. Their papers should be carefully studied by every worker who plans to use chemical methods.

The drawbacks of purely chemical methods, from the point of

view of clinical diagnostic procedures, are as follows: (1) they are laborious and time consuming—(the method I have described requires not less than a week for completion); (2) they are likely to give variable results when carried out intermittently because of the number and variety of procedures; (3) they require the constant supervision of a competent analytical chemist; (4) they result in loss of lead which, though uniform for the specific method, varies from method to method so that the findings of each must be interpreted on its own background; (5) they require facilities which are not generally available in diagnostic laboratories.

2. Electrolytic separation of lead from other metals

These methods combine chemical and electrical procedures and require a thorough knowledge and a considerable experience with both techniques. Early observations led me to reject electrolytic methods, and I have found no reason for returning to them. It would seem, however, that others have found a means of handling them satisfactorily. Reference should be made to the papers of Cooksey and Walton,[4] and to those of Weyrauch,[13] and Schmidt and Weyrauch.[9]

3. Radioactive indicators and electroscopic estimation of lead

This ingenious method is based upon the apparent fact that mixtures of radioactive lead and ordinary lead remain mixed in the same proportions throughout their career in the animal organism. The radioactive fraction present in a tissue or in excreta can be measured electroscopically and hence the total lead can be estimated. The method is only suitable for following administered lead and is therefore not generally applicable. The papers of Behrens[2] and others describe this method, which is based on the observations of von Hevesy.[6]

4. Estimation of lead through the use of diphenyl-thiocarbazone

This method, devised by Fischer,[5] has been used and reported on by others.[8, 10] It is based on the specific extraction of lead compounds from other metals in neutral solution, through the

formation of a colored compound with diphenyl-thiocarbazone in chloroform. It is exceedingly sensitive and according to its proponents can be used for the quantitative determination of amounts of lead as minute as 0.001 mg. As applied to biological materials, all that is required is the complete digestion of the organic compounds, neutralization of the resultant solution, quantitative extraction of all lead with an excess of the "dithizone" reagent in the presence of potassium cyanide, subsequent release of the lead with hydrochloric acid, and titration of the neutralized hydrochloric acid solution with carefully standardized "dithizone" in chloroform.

It has the advantages that it requires little equipment, few reagents, only a short time for its execution, and is applicable to small samples. I have not used it and cannot vouch for its accuracy.

5. Spectrographic determination of lead

I can recommend unreservedly this method for its accuracy, sensitivity, speed of manipulation, and the apparent universality of its application to all types of material. I resorted to its use for the study of samples that could not be handled by our chemical method, and after some six months of experience have found it very good. A description of the precise procedures of a standardized and simple method for the measurement of lead in urine will appear elsewhere soon.[3] The preparation of the sample, the excitation of the spectral lines, the photography, the development and reading of the plates and the calculation of results must be done with precision, but the whole process can be handled with dispatch and convenience if the proper equipment is available. The greatest difficulty lies in the expense associated with the use of a large spectrograph which will give a wide dispersion of spectral lines. It is, however, the most satisfactory method available for research purposes.

CONTAMINATION OF SAMPLES IN PROCESS OF COLLECTION AND ANALYSIS

The bugbear of all analysts of lead in biological materials is the ever present opportunity for the contamination of samples.

Lead is ubiquitous. Reagents of the highest purity show traces of lead. All glassware contains lead. Even filter paper and distilled water may have minute amounts of lead in them. Dust suspended in the air of the laboratory may contain significant amounts of lead especially when there are any painted surfaces, and when laboratory hoods have been surfaced on the inside with paints which have not been proved by analysis to be lead-free, the disintegrating surface will prove to be an important source of lead in the samples. The analyst must be on the lookout continually for such extraneous sources of lead, and despite the greatest care must carry blanks along with every set of samples to guard against their unrecognized presence. The difficulties here are as nothing, however, compared to the opportunities for contamination which occur during the collection of samples. Under prevailing hospital conditions it is impossible to obtain satisfactory samples for analysis. Needles and syringes of the usual type and sterilized in the usual manner always introduce lead into blood and spinal fluid. Bed pans and urinals frequently provide gross contaminations. Urine samples taken with a rubber catheter may give wholly worthless results. The customary necropsy technique is almost certain to result in contamination of tissues. In fact any sample which has come in contact with any material other than that which has been carefully selected and cleaned for the purpose must be regarded as unsatisfactory and likely to give a misleading result. Moreover, the activities of the patient during the period preceding and during the collection of the sample must be known. A dose of Epsom Salts will spoil a fecal sample because of the lead content of the salt commonly employed.* The drinking of unusual amounts of water may obscure the urinary findings, while other therapeutic measures to which the patient may have been subjected may so change the distribution of lead in the tissues and excreta as to yield abnormal results. It may not be entirely out of place at this juncture to caution against the interpretation of results which have been obtained after the production of an ammonium-chloride or other therapeutic acidosis. Some students

* As little as 0.01 per cent lead in commercial or U. S. P. $MgSO_4$ will contribute 1.5 to 3.0 mgm. of lead per 15-gram or 30-gram dose of this salt.

of the problem have attempted to promote lead excretion by various means and have regarded any elevation of the excretory rate as evidence of the presence of unusual amounts of lead in the tissues of the patient. This, I believe, is unsound. In my experience, the best measure of the extent of the lead absorption of a live man is to study the lead of his blood, and the lead content of his excreta under the most nearly physiological conditions.

AMOUNT OF SAMPLE IN RELATION TO SENSITIVITY OF ANALYTICAL METHOD

It seems obvious that the limitations of an analytical method with regard to its sensitivity must be the factor which determines the size of the samples to be employed. Nevertheless, since this point has not received the attention it deserves, it may be well to cite an example which will demonstrate its importance.

The chemical method employed in our laboratory yields a substantially uniform loss of approximately 0.07 mgm. of lead per sample. The range of normal lead excretion, established by the analysis of three- and four-liter samples by this method, runs from 0.01 to 0.05 mgm. per liter. Most normal urines in amounts of a liter or less will give negative results. On the other hand, the urine of severely exposed persons, immediately after the cessation of exposure rarely contains lead in excess of 0.30 mgm. per liter. Moreover the rate of urinary lead excretion drops off after cessation of exposure so that frequently by the time the urine has been obtained for examination, it has little more than 0.15 mgm. per liter. A liter of such urine will yield only 0.08 mgm. of lead on chemical examination by our method, and if only half a liter is examined, it will show only traces of lead. A satisfactory basis for a diagnosis will not have been furnished by such findings, whereas with a large sample the evidence of significant lead absorption would have been provided.

THE INTERPRETATION OF RESULTS

Every analytical method has its own limitations which are best appreciated by the person most experienced in their use, and just as the qualitative detection of lead in tissues or excreta is

worthless, so also are analytical results without an interpretation founded upon a background of observations covering the field into which the results must be fitted. Results obtained by one method can not be compared directly with those obtained by another method unless the difference in sensitivity of the two methods is known. By way of illustration, the lead concentration in the blood of normal persons as determined by the spectrographic technique referred to above ranges from 0.01 to 0.12 mgm. per 100 cc., with a mean value of 0.06, whereas by the chemical method it ranges from nil up to 0.06 mgm. per 100 cc., the amounts usually being too small to estimate quantitatively when 50 cc. samples of blood are analyzed. By the latter method any result above 0.06 must be regarded as pathological, whereas such a result is well within the normal range if obtained spectrographically. It is clear, therefore, that until the several methods employed by laboratory workers have been standardized to the point where they yield consistent results which can be translated into absolute terms, only confusion can result from their use in diagnostic laboratories.

If the foregoing paragraphs are interpreted as an attempt to discourage the attempts of laboratory workers in general to carry out lead analyses as diagnostic measures, they will not have been misconstrued. On the other hand there can be no doubt that such analyses, properly carried out on properly collected materials, contribute useful and in many instances vital information. Under suitable circumstances the line of demarcation between normal and abnormal is distinct, whether dependence is put upon analysis of blood, excreta or tissues. In the case of the tissues it is advisable to obtain representative and adequate samples of the various organs so that the approximate total lead in the body may be estimated. In the present state of our knowledge, no interpretation can be made of results which do not include representative portions of skeleton, muscle, and liver. Other organs, especially blood, lungs, spleen and kidneys, should be analyzed if possible. Obviously, also, in suspected central-nervous-system lead intoxication the brain and cord should be examined.

REFERENCES

(1) Avery, D., Hemingway, A. J., Anderson, V. G., and Read, T. A.: Determination of minute amounts of lead in water, with notes on certain causes of error. Proc. Australasian Inst. Mining Met., n. s., **43:** 173–199. 1921.

(2) Behrens, B., and Behrens, H. O.: Zur Pharmakologie des Bleies. Arch. f. exper. Path. u. Pharmak., **164:** 501–508. 1932.

(3) Cholak, J.: The quantitative spectrographic determination of lead in urine. Jour. Am. Chem. Soc., In Press.

(4) Cooksey, T., and Walton, S. G.: Electrolytic determination of lead in urine. Analyst., **54:** 97–99. 1929.

(5) Fischer, Hellmut, and Leopoldi, Grete: [The quantitative microchemical determination of heavy metals with the use of dithizone (diphenylthiocarbazone)]. Wiss. Veröff. a. d. Siemens-Konzern, **12:** 44–52. 1933.

(6) v. Hevesy and Paneth: Lehrbuch der Radioaktivität. Leipzig, p. 107, 1923. Cited by Behrens.

(7) Kehoe, R. A., Thamann, Frederick, and Cholak, J.: On the normal absorption and excretion of lead. Jour. Ind. Hyg., **15:** 257–272. 1933.

(8) Ross, J. R., and Lucas, C.: A new method for the detection and estimation of minute amounts of lead in biological materials; a preliminary report on recovery of lead from cases of lead poisoning. Canad. Med. Assn. Jour., **29:** 649–650. 1933.

(9) Schmidt, Paul, and Weyrauch, F.: Über die Diagnostik der Bleivergiftung im Lichte moderner Forschung. Jena: Gustav Fischer, 1933, pp. 76.

(10) Seelkopf, K., and Taeger, H.: Quantitative Bestimmung kleiner Bleimengen. Ztschr. f. d. ges. exper. Med., **91:** 539–548. 1933.

(11) Tannahill, R. W.: A critical survey of the methods for the determination of lead in biological material. Med. Jour. Australia, **1:** 194–201. 1929.

(12) Taylor, H. B.: The determination of minute quantities of metals in biological material. Jour. Proc. Roy. Soc. New So. Wales, **61:** 315–336. 1928.

(13) Weyrauch, F.: Neue Untersuchungen über die Aufnahme des Bleies und seine Verteilung im Organismus bei experimenteller Vergiftung. Ztschr. f. Hyg. u. Infektionskr., **111:** 162–179. 1930.

STUDIES OF EDEMA, ESPECIALLY THE EDEMA OF RENAL ORIGIN

ESLEY J. KIRK

From the Department of Biochemistry, University of Nebraska; College of Medicine, Omaha, Nebraska

The classification of edema is usually based upon the underlying pathological condition associated with its formation.

This study is chiefly concerned with the non-inflammatory edemas, the fluid of which is thin, transparent and clear, colorless or of a slightly yellow color, of low specific gravity (below 1.018), low protein content and does not coagulate spontaneously. Since this is the type of edema that is commonly associated with diseases of the kidneys, we shall limit our study almost entirely to the so called renal edemas.

The manner of edema formation in various pathological conditions has not been clearly or definitely explained. In relation to the edemas of renal origin the kidneys were thought to be the main and possibly only factor. It is only recently that factors other than the kidneys have been found that influence edema formation more than the kidneys themselves.

HISTORICAL

The study of edema in relation to the kidneys dates back many years and has resulted in a number of hypotheses. Even today, after many years of intensive investigation, the correlation of renal pathology and edema formation is not yet clearly understood, and little has been added regarding the fundamental nature of nephritic edema since the work of Bright. Gradually, certain modifications of Bright's work and, in other instances, more detailed and confirmatory knowledge has been added.

Bright associated albuminuria with edema formation. He observed that the serum proteins were decreased as the result of protein loss through the urine. He thought that an hydremia

resulted from kidney damage and that the blood, owing to the loss of its albumin through the kidneys, allowed its fluid portion to filter more easily through the walls of the small vessels. Johnson, Bartels, and Stewart supported the hydremic plethora theory but they also promulgated the idea that there existed an increase in vascular pressure which, owing to an insufficient elimination of water by the kidneys (oliguria), caused the edema.

The idea of Bright, that hydremia is the underlying cause, prevailed with slight modifications until 1895. At this time Senator proposed the hypothesis of increased permeability of the capillaries, under the influence of toxic agents of various nature, as the cause of edema. Albuminuria, which is the result of serum protein escaping through the glomerular capillaries, exemplified this increased permeability. Its existence was supported by demonstrable renal pathology. Previously Rees had also stated his belief that edema was due to a toxic dyscrasia, the kidney ceasing to excrete injurious substances. Heidenhain, who developed the theory of the excretory nature of lymph formation, attempted to identify the nature of these toxins. He investigated the properties of lymphogenous substances that were capable of provoking edema. Heidenhain considered edema formation as an excessive secretion of lymph. The study of kidney toxins known by various terms such as "nephrotoxine," "nephrolysins" and "nephroblaptins," however, has not been fruitful.

Following Senator, Starling advocated that fluid escaped through the capillary membrane as the result of changes in the colloid osmotic pressure of the plasma proteins. He stated that a delicate balance existed between the hydrostatic pressure in the capillaries and the osmotic pressure of the plasma proteins. Csatasy a few years previously had noted that a decrease in the plasma proteins occurred in some patients with nephritis and that the (serum) albumin was lowered more than the (serum) globulin. Virchow had already suggested that edema was due to the attraction of water by the tissues as a consequence of nutritional disturbances.

It was at this period of the study of edema formation that a differentiation between the various types of edema, especially

those related to renal pathology, was instituted. This was, primarily, the result of Starling's work which was accepted by many.

During the period from Bright to Starling (1830 to 1896) frequent reference had been made to water and salt retention by the kidneys as a cause of edema formation. Bartels and Stewart taught that the elimination of fluid through the kidneys was retarded, resulting in an hydremia. Winter showed that the constancy of the molar concentration of the blood was maintained by the exchange of water and chloride molecules between plasma and tissue. He thought that the sodium chloride was more important in edema formation than changes in the osmotic pressure of the proteins. Bright advocated that hydremia was the result of kidney damage, but that the formation of edema was due to the depletion of the blood proteins following albuminuria. It was not, however, until 1901 that the cause of edema from salt retention was actually recognized. This was mainly the result of the work of Achard, Strauss and Widal. Strauss and Widal showed that production of edema and its severity varied with the salt intake. On the basis of this observation these authors used a diet poor in salt for the treatment of edema. Reichel had found that edematous swelling produced by the injection of saline persisted longer in cases of Bright's disease than in other types of affection. Cohnheim and Lichtheim, and Magnus had produced dropsy by inundating the organism with large quantities of salt solution of different concentrations. Chaufford noticed the occurrence of edema of the face in a jaundiced patient treated by repeated injections of saline solution. Such and many similar observations led these workers to elaborate the chloride retention hypothesis of edema formation, the failure of the kidneys to excrete chlorides being considered the mechanism.

Fischer[6] as early as 1910 attempted to explain edema on the basis of colloidal chemical changes in the tissues. He thought that the cause of edema resided in the tissues themselves, which became edematous not because water was forced into them by osmosis, but because they suffered changes which increased their

swelling capacity. He showed that severe edema may be produced in the absence of circulation. He believed that the colloidal chemical changes in the tissues are due to an abnormal production or accumulation of acid products. Fischer maintained that the edematous fluid in serous cavities was due to a "squeezing off" effect of the proteins in the tissue. In support of this view he relied upon the phenomenon of separation of a thin colloidal solution from hydrated solid colloid upon standing (syneresis).

Achard, Ribot and Leblanc[1] noted a change in the ability of body fluids and tissues to hold water in relation to the lipoids. They based this on the observations of Mayer and Schaeffer, who found that the ability of tissues to absorb water was proportional to their lipocytic index, expressed by the ratio, cholesterol: fatty acids. In studying this lipocytic index in different cases of dropsy, Achard et al. found higher figures in cases of Bright's disease with edema than in cases without edema or in cases with edema of a cardiac origin. They found that neither fatty acids nor cholesterol were miscible with water and that fatty acids resisted cellular imbibition of fluid, but the more cholesterol there was present the less this resistance became.

Elwyn[5] advanced the hypothesis that edema formation was due to a disturbance of the central nervous control of water balance. He was unable to explain edema formation resulting from changes of hydrostatic pressure or plasma protein osmotic pressure. He is of the opinion that no physico-chemical system can alone explain the entire mechanism of any vegetative function, such as water balance of an organism.

The difficulty in explaining edema formation is that one often attempts to interpret the changes on the assumption that only one mechanism is operative, yet the following factors may influence edema formation: (1) capillary hydrostatic pressure, (2) colloid osmotic pressure of the blood proteins, (3) salt retention in the tissues, (4) specific tissue changes, (5) permeability of vessel wall, (6) lymphatic drainage, and (7) nervous and hormonic control.

Barker and Kirk[2] have previously studied the problem of

edema in patients with marked persistent generalized edema and in dogs with the edema produced by plasmapheresis. The summary of the results of study on these patients can be briefly stated as follows: the greater the albuminuria, the lower the serum proteins, and the lower the serum proteins, especially the albumin fraction, the greater the edema. The studies on dogs showed that edema formation occurred with the lowering of serum proteins, especially the albumin fraction.

The following experimental studies on dogs were done in order to reveal the concomitant changes, such as pH, changes of the serum and urine, together with changes of the blood, gastric and urinary chlorides.

METHOD OF PROCEDURE

Healthy dogs were selected and subjected to preliminary study. Plasmapheresis was the method adopted to produce low proteinemia. The dogs were bled at intervals for periods varying from 11 to 19 days. The total amounts of blood removed varied from 6,620 to 10,090 cc. The bleeding was done by puncture of the femoral arteries or heart, and the re-infusion through a vein. All procedures were carried out with aseptic technique so that there never was any infection. During experiments the animals were maintained on a definite nitrogen intake, and a sufficient amount of fluid was allowed.

The total serum protein and the albumin and globulin determinations were made by the combined methods of Howe, Wu, and Koch and McMeekin, as described by Hawk and Bergeim.[8] The nitrogen of the diet was estimated by the macro-Kjeldahl method, and the nitrogen of the urine was estimated by the micro-Kjeldahl method as described by Hawk and Bergeim. The chlorides of the blood and of the urine were estimated by the methods of Whithorn and Volhard-Arnold. The urinary acidity titration was made according to the method described by Morgulis and Hamsa.[10] The pH of the blood serum was determined electrometrically, using the quinhydrone electrode described by Cullen and Büllmann,[3] and Cullen and Earle.[4] The gastric contents were collected by means of a Rehfuss stomach tube and determinations were made of the chlorides, and of the free and total acidity by titration with 0.1 N sodium hydroxide.

PROTOCOLS

Dog 1, a full grown bull weighing 23.1 kgm., was studied over a control period of ten days. The daily diet and the various chemical values during the control period may be observed in table 1.

Plasmapheresis was started on the 11th day and continued for twelve days. It was discontinued at that time because of the onset of diarrhea. On the 27th

TABLE 1

Dog 1

Day	Body weight	Nitrogen intake	Urine				Blood						Gastric juice		
										Proteins				Acidity	
			Volume	N	Cl	Acidity 0.1N	pH	Non-protein nitrogen	Cl	Total	Albumin	Globulin	Cl	Free	Total
	kgm.	gm.	cc.	gm.	gm.	cc.		mg. %	mg. %	%	%	%	mg. %	cc.	cc.
0[1]	24.14	14.6													
1	24.13	14.6	1,408			207.6									
2	23.59	14.6		12.0	9.05			38.2	472						
3		14.6													
4	23.59	14.6	1,080	12				35.8		6.2	3.2	3.0		9.20	15.5
5		14.6	1,080	10.2			7.32		499	6.2	3.6	2.6	134.5	13	25.9
6	23.33	14.6													
7	23.89	14.6	1,400	8.4	5.35	131									
8		14.6	1,220	7.5	4.95	152									
9	23.96	14.6	1,500	7.85	5.78	155.4	7.32								
10		14.8	1,700	7.2	6.5	142.6									
11[2]	23.96	11.0	1,700	7.2	6.5		7.32			5.8	3.5	2.3	117	4.84	10.87
12[3]	23.98	9.6							516						
13[4]	24.43	9.6					7.32			4.8	3.1	1.7			
14[5]	23.64	9.6							503	3.3	2.6	0.7			
15[6]	24.04	4.8						26.6	502				111	0	6.9
16[7]	25.52	9.6	2,100	4.7	6.4	45.8	7.35			3.5	2.1	1.4	132	2.3	7.7
17[8]	24.8	7.6	1,490				7.35		528					0	20.9
18[9]	23.43	5.7	1,200							3.5	2.3	1.2	125		
19	22.99	5.7	1,300						540						

[1] The diet during the control period consisted of 1,000 cc. of milk, 480 cc. of egg white, 35 grams of sugar and 4.6 grams of salt mixture. (0.63 gram of nitrogen per kgm. of body weight.)

[2] Plasmapheresis of 1,075 cc. of blood. The nitrogen intake was decreased to 9.6 grams per day or 0.42 gram per kgm. body weight.

[3] Plasmapheresis of 1,000 cc. of blood.

[4] Plasmapheresis of 1,450 cc. of blood. Diarrhea.

[5] Plasmapheresis of 450 cc. of blood.

[6] Edema noted of extremities; definite pitting. Transfusion of 275 cc. of blood, later followed by plasmapheresis of 1,175 cc. of blood.

[7] Plasmapheresis of 900 cc. Edema present.

[8] Plasmapheresis of 450 cc. Edema present (2+).

[9] Nitrogen intake decreased to 0.25 N/kgm. of body weight. Edema 3+. Plasmapheresis of 850 cc.

TABLE 1—*Concluded*

DAY	BODY WEIGHT	NITROGEN INTAKE	URINE				BLOOD						GASTRIC JUICE		
			Volume	N	Cl	Acidity 0.1N	pH	Non-protein nitrogen	Cl	Proteins			Cl	Acidity	
										Total	Albumin	Globulin[1]		Free	Total
	kgm.	*gm.*	*cc.*	*gm.*	*gm.*	*cc.*		*mg. %*	*mg. %*	*%*	*%*	*%*	*mg. %*	*cc.*	*cc.*
20[10]		5.7						34.7	603					8.79	14.78
21[11]	23	5.7					7.29						111.2		
22[12]	23	5.7					7.28		548	4.9	2.6	2.3			
23	22.46	5.7													
24		5.7													
25		5.7	800	3.1											
26		5.7													
27[13]	22.8	5.7													
28[14]		5.7		2.1	4.94			38.4	482	5.6	3.2	2.4			
29[15]	22.78	5.7	245		3.52		7.32						122.5	4.9	21.4
30[16]		0	1,200	2.5	3.9		7.28			4.2	2.5	1.7			

[10] Erythrocytes, 6,500,000; leukocytes, 10,300; plasmapheresis of 450 cc.

[11] Plasmapheresis of 450 cc.

[12] Plasmapheresis of 450 cc. Following 4 days plasmapheresis discontinued because of diarrhea.

[13] Plasmapheresis of 400 cc.

[14] Plasmapheresis of 650 cc.

[15] Plasmapheresis of 400 cc.

[16] Plasmapheresis of 450 cc. Sudden death.

day, or five days later, plasmapheresis was again resumed and was continued to the 30th day, when the dog died. The cause of death was thought to be collapse and shock from bleeding. The dog showed poor tolerance for bleeding which manifested itself in restlessness, extreme weakness and slow deep respirations. The pH of the blood dropped to 7.28. Slight diarrhea and vomiting occurred. The nose was dry before bleeding started, though the dog appeared to be in excellent condition. Before death the serum appeared milky.

As plasmapheresis was begun, the nitrogen of the diet was diminished. The nitrogen output in the urine also decreased. The urinary chlorides for the control period averaged 6.2 grams per day, but during the period of bleeding the average daily output of chlorides was 4.7 grams. The blood chlorides increased during the period of plasmapheresis; there was a marked change in the protein content of the serum (see fig. 1). The total amount of blood removed during the plasmapheresis was 8,750 cc. or a daily average of 777 cc. Up to the appearance of edema, which occurred on the 15th day (5th day of bleeding), the daily average bleeding was 1,030 cc. The chloride content

of the gastric juice dropped from 134 mgm. per cent to as low as 111 mgm. per cent. No remarkable change was noted in the pH of the urine, unless the urine became more acid, possibly due to the diet. The free and total acidity of the gastric juice showed a definite decrease as compared with the findings made during the control study period.

Dog 3, a brindle bull, weighing 19.4 kgm., was studied over a control period of 10 days. The diet was essentially the same as for Dog 1, but contained 16 grams of nitrogen per day. The values may be seen in table 2 for the control period.

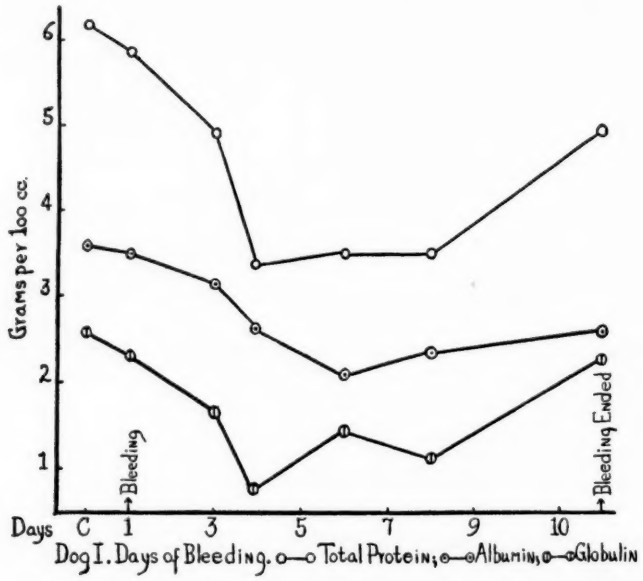

FIG. 1

Plasmapheresis was started on the 11th day, and edema appeared on the 16th day. During the entire plasmapheresis period 7,800 cc., or an average of 410 cc. per day, were removed. The weight of the dog increased to 20.5 kgm. during the edema stage. The urinary nitrogen diminished (table 2); and the urinary chlorides decreased. During the latter stage of edema the serum pH was lowered to 7.34 which is only slightly more acid than during the control period. No noteworthy change occurred in the non-protein nitrogen of the blood but the serum proteins as well as the albumin and globulin fractions changed remarkably, as is illustrated in figure 2. The blood chlorides increased during the edema stage (see fig. 3). The pH of the urine remained as was found during the control period. The free and total acidity of the gastric contents decreased markedly during plasmapheresis.

TABLE 2

Dog 3

DAY	BODY WEIGHT	NITROGEN INTAKE	URINE			BLOOD						GASTRIC JUICE		
			Volume	N	Cl	pH	Non-protein nitrogen	Cl	Proteins			Cl	Acidity	
									Total	Albumin	Globulin		Free	Total
	kgm.	*grams*	*cc.*	*grams*	*grams*		*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*cc.*	*cc.*
1[1]	19.0	16	200	2.2	3.1	7.40	39.6		6.4	4.5	1.9	152	31.6	45.0
2		16						457	6.1	4.2	1.9			
3		16				7.40			6.1					
4	19.3	16	300	14.6	5.2									
5		16												
6		16	550	14.3	3.74									
7		16												
8		16												
9		16												
10		16	1,400	15.2	6.70	7.40							42.0	65.8
11[2]	19.5	16												
12		16												
13	19.4	16					39.3	448						
14		16										142	41	7.9
15[3]		16												
16	19.7	16	625	13.9	5.3	7.41	41.6	459	4.8	3.0	0.8			
17		16	600											
18	20.1	16		8.6	2.7									
19	20.4	13.5				7.40		502	4.2	2.2	1.9	128	18.5	28.7
20	20.3	18												
21	20.5	16					40.2	540						
22		16				7.34						130	4.8	24.0
23		16						540	5.2	3.2	2.0			
24	19.3	16					39.2	561	3.5	2.4	1.1			
25	19.9	16	750	8.3	5.2									
26	19.6	16	1,150	12.4	12.23	7.34								
27	19.5	16				7.34	37.2	561	4.4	3.3	1.1	147		
28		16												

[1] The daily diet consisted of milk, egg white, butter and lard mixture, sugar and salt mixture, so that 16 grams per day or 0.8 gram of nitrogen per kgm. of body weight were given. Preliminary period lasted 10 days.

[2] Plasmapheresis started and continued over a period of 18 days with a daily average bleeding of 410 cc.

[3] Edema of the extremities appeared on this day. It increased and persisted during the remaining period of plasmapheresis, i.e., up to the 29th day. At this time the animal refused the food and no more blood was drawn.

TABLE 2—*Concluded*

DAY	BODY WEIGHT	NITROGEN INTAKE	URINE			BLOOD						GASTRIC JUICE		
			Volume	N	Cl	pH	Non-protein nitrogen	Cl	Proteins			Cl	Acidity	
									Total	Albumin	Globulin		Free	Total
	kgm.	*grams*	*cc.*	*grams*	*grams*		*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*cc.*	*cc.*
29[4]	19.4	0										135	10	16
30		0												
31		0												
32	19.4	9	1,500	15.4	8.89	7.41	38.0	501	4.9	3.5	1.4			
33		24												
34		24												
35		24												
36	19.3	18		22.7		7.44								
37	19.5	18	800	23.5	14.8		44.6	499	5.6	3.1	2.5			

[4] Last bleeding.

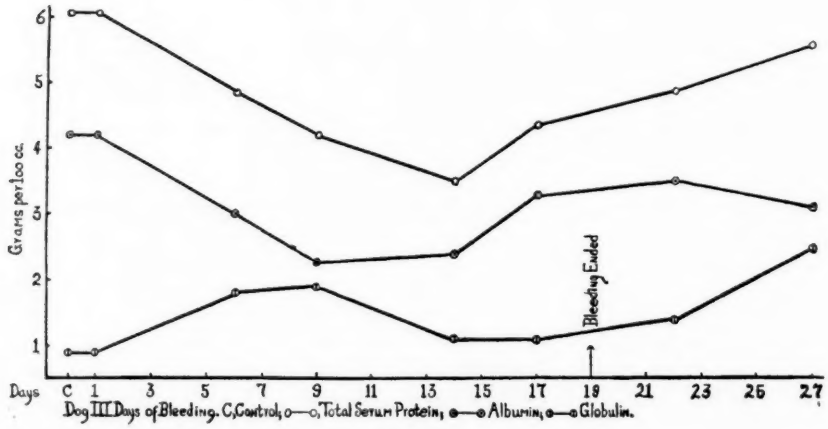

FIG. 2

Dog 4, a male shepherd, weighing 22.4 kgm., was fed a diet consisting of hamburger, butter and lard, sugar, and salt mixture. The intake of nitrogen was 12 grams per day. The dog ate the diet every day except the 14th, 15th and 16th days when he refused to eat or ate only part of the food. Table 3 gives the various chemical values for the control period.

On the 7th day plasmapheresis was begun, the total amount of blood removed during this period being 6,630 cc. or an average of 735 cc. daily. The

erythrocyte count at the beginning of the period was 6,500,000; on the 16th day of the experiment the red blood count was only 3,940,000. A blood transfusion was done and the following day the blood count was 5,430,000 erythrocyte

TABLE 3

DOG 4

DAY	BODY WEIGHT	NITROGEN INTAKE	URINE			BLOOD						GASTRIC JUICE		
			Volume	N	Cl	pH	Non-protein nitrogen	Cl	Proteins			Cl	Acidity	
									Total	Albumin	Globulin		Free	Total
	kgm.	*grams*	*cc.*	*grams*	*grams*		*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*cc.*	*cc.*
1		16.5												
2		16.5												
3		13.8												
4	22.2	12	960	12.8	9.9		38.7	490.8	6.2	4.2	2.0			
5		12				7.40						135	0	13
6	21.6	12				7.43						175	2.7	4.4
7[1]		12				7.37		499.2				61	1.7	3.6
8	21.4	12												
9		12	1,170	11.5	8.9									
10	21.5	12	1,310	9.8	9.7	7.37		512	4.3	3.0	1.3	411	16.9	63.0
11		12		10.4	11.3	7.38								
12		12	3,380	10.4	11.3	7.40								
13		12	1,000	6.4	8.6			560						
14	21.6	0				7.38						156	0	4.3
15		0				7.31								
16[2]	20.9	6	2,750	11.2	14.2				4.9	2.4	2.5			
17[3]	20.3							500						

[1] Control period ended and plasmapheresis began. The diet consisted of hamburger, butter and lard, sugar and salt mixture.

[2] Period of plasmapheresis ended. Total number of cubic centimeters of blood removed was 6,630 or a daily average of 735 cc. The animal was doing poorly. The erythrocyte count had dropped from 6,500,000 to 3,940,000. Transfusion was done with return of blood count to 5,430,000.

[3] Accidental death due to myocardial puncture.

and 10,700 leukocytes. The dog died on the 17th day accidentally, and a necropsy revealed blood in the pericardial sac due to a myocardial puncture.

The values during the period of plasmapheresis may be seen in table 3. The blood chlorides increased to as high a level as 560 mgm. per cent. The average blood chloride for the period was 518 mgm. per cent. There was no demon-

strable edema nor any appreciable change in the pH of the blood in this dog. The gastric chlorides decreased except on one occasion when they were found to be 411 mgm. per cent. The free and total acidity of the gastric secretion was low throughout both periods of study with the exception of an increase that occurred simultaneously with the high chloride content.

Dog 6 weighed 24.9 kgm. The preliminary study showed a blood pH of 7.38, chlorides 508 mgm. per cent, non-protein nitrogen 39 mgm. per cent, total serum protein of 6.9 grams per cent with 4.5 per cent albumin and 2.4 per cent globulin. The dog was fed meat and vegetables.

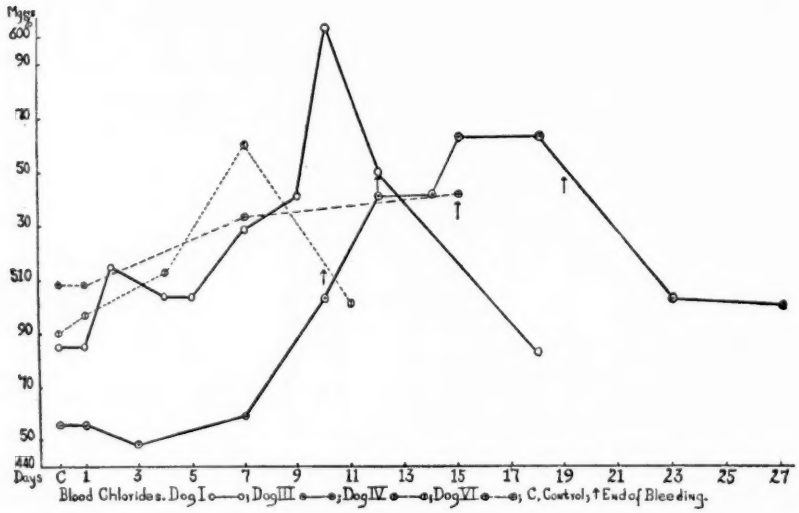

FIG. 3

Following the control period intensive plasmapheresis was carried out, 10,090 cc. of blood being removed or a daily average of 840 cc. On the 7th day of bleeding the pH of the blood was 7.46, the chlorides 533 mgm. per cent and the total protein of the serum 4.4 per cent, 2.5 per cent albumin and 1.9 per cent globulin. The body weight of the dog dropped to 23.7 kgm. On the 10th day the weight had increased to 24.2 kgm. The pH of the blood serum was 7.40. On the 13th day the weight had increased to 25.1 kgm. The pH of the blood dropped to 7.34. On the 15th day the blood chlorides had increased to 544 mg. per cent and the total protein dropped to 4.2 per cent; 2.0 per cent albumin and 2.2 per cent globulin. On the 16th day the weight reached 25.3 kgm. The blood pH was 7.35. On the 17th day the dog died as a result of myocardial puncture.

COMMENTS

I studied in dogs the alterations of the blood, gastric contents, and urine together with certain objective findings accompanying plasmapheresis. The changes may be outlined as follows:

Blood
1. Low proteinemia
2. Hyperchloremia
3. Increase of hydrogen ion concentration

Urine
1. Decrease of chlorides
2. Decrease of nitrogen

Gastric contents
1. Hypochlorhydria to complete achlorhydria
2. Decrease of chlorides

Objective findings
1. Gain in weight
2. Loss of appetite
3. Diarrhea
4. Edema formation

The average daily removal of from 410 to 841 cc. of blood with subsequent reinfusion of the cells resulted in a lowering of the proteins of the blood. At no time during the period of plasmapheresis was there a definite inversion of the ratio of albumin to globulin. This may possibly have been due to the fact that egg white supplied a considerable part of the protein of the diet. The dogs, however, were placed on a low protein diet during the periods of plasmapheresis. Dog 1 during the control period received 89 grams of protein per day. When plasmapheresis was started the protein intake was lowered to 59.8 grams per day and on the 8th day of this period the protein intake was again lowered to 35.7 grams per day. On the 5th day of plasmapheresis, during which time an average of 1,030 cc. was removed daily, a definite edema of the legs was noted which persisted for six days. Unfortunately, at this time a severe diarrhea developed which prevented metabolic studies and plasmapheresis was discontinued. Edema occurred as the total serum proteins fell to a level of about 3.5 grams per cent (See fig. 1). The serum albumin fraction ranged from 2.1 to 2.6 and the globulin fraction from

0.7 to 1.4 grams per cent. At the time edema appeared there was an increase in weight of 1.4 kgm. (See fig. 4). Edema occurred in dog 3 when the serum protein level was between 3.5 and 4.8 grams per cent (fig. 2). The serum albumin and globulin fractions ranged from 2.3 to 3.0 and 1.1 to 1.9 grams per cent, respectively. There was also an increase in body weight of about 2 kgms. during the edema period (fig. 4). Edema was not noted in dogs 4 and 6. In dog 4 the total serum protein did not fall below 4.3 grams per cent. The albumin fell to 2.4 and the

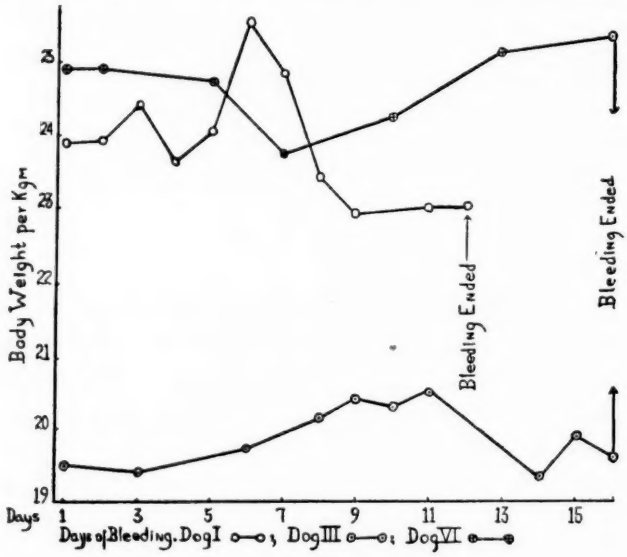

FIG. 4

globulin to 2.5 grams per cent. This dog was on a protein intake of 75 grams during the period of plasmapheresis, but was not fed by stomach tube as dogs 1 and 3. The body weight decreased by 2 kg. during the period of bleeding. The dog died accidentally before the experiment was completed. In dog 6 the total serum protein fell as low as 4.2 grams per cent while the serum albumin and globulin fractions were as low as 2.0 and 1.9 grams per cent respectively. This dog was not fed a definite diet, but was allowed to eat as much as he wanted. His appetite, however, was markedly reduced during the period of plasmapheresis as was

also the case in all of the dogs. Although no gross edema was noted in dog 6, there was, nevertheless, a gradual increase in weight (fig. 4) and the skin was of a doughy consistency.

The result of the plasmapheresis in the dogs together with a low protein diet was a lowering of the serum proteins. I am of the opinion that the continuous loss of serum proteins produces a lowered colloid osmotic pressure of the blood which is in accordance with Starling's hypothesis of edema formation. As a result the amount of water moving from the capillaries to the tissues would increase and the rate of movement of water from the tissues to the capillaries would decrease. There is no reason to believe that any change occurred in the filtration pressureof the capillaries. In fact studies that have been previously described show no change in the filtration pressure of the capillaries in cases of prolonged albuminuria with edema (nephrosis). That no increased permeability of the capillaries occurs is also assumed and this is based upon studies of the blood, of ascitic and edema fluid, as previously described.

An interesting feature of the findings in dogs undergoing plasmapheresis was the changes in blood and gastric chlorides. Parallel with the development of a low serum protein occurred a striking increase of the blood chlorides (fig. 3) and decrease of the gastric chlorides. The blood chlorides of dog 1 during the control study period ranged from 472 to 499 mgm. per cent. In the period of plasmapheresis the blood chlorides gradually increased from 516 to as high as 603 mgm. per cent. The chloride content of the gastric fluid was 134.5 mgm. per cent during the control period and decreased to as low as 111 mgm. during plasmapheresis. The free and total acidity of the gastric fluid also decreased greatly during plasmapheresis, or free acid disappeared entirely and the total acid diminished to only 6.9 cc. The urine volume output during plasmapheresis remained about the same as before bleeding, with occasional diuresis. The chloride output in the urine remained about the same in the control and bleeding period. In dog 3 the blood chlorides during the control period ranged from 457 to 468 mgm. per cent. During plasmapheresis the blood chlorides rose to 561 mgm. per cent

and returned to normal when bleeding was discontinued. The gastric chlorides for the control period was 152 mgm. per cent and decreased to 128 during bleeding. The free acid content of the gastric fluid ranged from 31.6 to 42.0 and total acid from 45.0 to 65.8 during the control period. During plasmapheresis the free and total acid of the gastric content decreased to as low as 4.1 and 7.9 respectively. Very little change of the chloride output in the urine occurred during plasmapheresis.

In dog 4 the blood chlorides increased from 499 during the control period to 560 mgm. per cent during the period of bleeding (fig. 3). The gastric chlorides during the control period ranged from 175 to 185 mgm. per cent. On the first day of bleeding (1500 cc. of blood) the gastric chlorides were 61 mgm. per cent; on the 4th day of bleeding the gastric chlorides increased markedly to 411 mgm. per cent with a corresponding increase of the free and total acid. On the 8th day of bleeding the gastric chlorides decreased to 156 mgm. per cent, with 4.3 cc. total acid and no free acid. The urine chlorides increased during the bleeding period.

It is probable that as the protein content of the blood decreased, chlorides entered the blood stream in accordance with Donnan equilibrium aiding to maintain a normal osmotic relation so important in water balance regulation. The lowering of the chlorides of the gastric secretion may be considered an attempt at the conservation of chlorides for this purpose.

Gilman and Cowgill[7] in dehydration experiments on dogs accomplished by depriving the animals of water for three day periods, found a marked increase of the blood and gastric chlorides. Their interpretation was that some osmotic relationship existed between the blood and the gastric juice. In my experiments the blood chlorides increased and the gastric chlorides decreased; I am unable at this time to offer an explanation for this.

The finding of low free and total acidity of the gastric fluid in the dogs during plasmapheresis can perhaps be explained by its decreased chloride content. MacLean and Griffiths[9] found that the chloride content of the gastric juice is very nearly constant. As it is secreted, part is unchanged as sodium chloride and part

is converted into hydrochloric acid which governs the acidity of the secreted gastric juice.

It was expected that the dogs would develop a negative nitrogen balance during the removal of serum and during the administration of a diet below maintenance in respect to proteins. The occurrence of diarrhea so common in dogs on artificial diets and especially common with the removal of large amounts of blood probably accounted for the low urinary nitrogen obtained in the studies. Considerable nitrogen was probably lost in the stools.

Diarrheas are frequently noted in patients with albuminuria and edema formation. This may perhaps be accounted for by

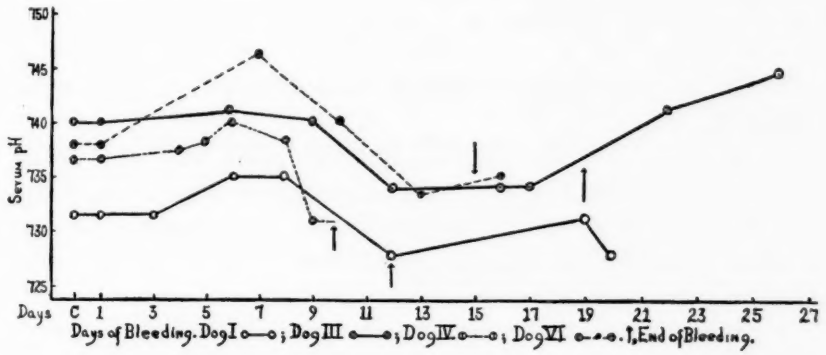

FIG. 5

the development of edema of the intestinal wall. This may also be the explanation of the diarrhea that occurred in the dogs. The study of the pH of the serum revealed no striking changes from the normal. The determinations of the pH of the serum of nephritic patients in the University Hospital has revealed changes comparable to the variations found in the normal. Although the CO_2 volume per cent of the blood plasma was below normal, no change occurred in the pH. CO_2 values of 34 to 44 per cent were not unusual in the nephritic patient. The lowest value of the CO_2 volume per cent found in the dogs during plasmapheresis was 44. The pH of the serum at that time was 7.40.

In dog 1 there was a slight rise in the pH during the edema

stage followed by a lowering of the pH when diarrhea set in, and just before death from shock (pH 7.28). In dog 3 no change occurred in the pH of the serum during the first stage of the edema, but in the second half of the period the pH dropped to 7.34. With the cessation of plasmapheresis the pH returned to its original level of 7.40. Dog 4 had a blood pH of 7.40 to 7.43 before bleeding, but, during the period of plasmapheresis, the pH dropped, first to 7.37 and finally to 7.31. Dog 6 showed a serum pH 7.38 before bleeding. On the fifth and eighth days of plasmapheresis the pH was 7.46 and 7.40 respectively. However, during the last four days of bleeding the pH dropped to 7.34 and 7.35 (see fig. 5).

CONCLUSIONS

(1) A definite low proteinemia was produced in dogs by plasmapheresis.

(2) Inversion of the albumin-globulin ratio did not occur. The lowering of the serum albumin fraction occurred at a greater rate than of the serum globulin fraction. The regeneration of the serum globulin occurred at a greater rate than of the albumin fraction after plasmapheresis was ended.

(3) Edema formation was associated with lowered serum protein. This was manifested by pitting edema of the legs in two of the dogs and by the doughy character of the skin in the others together with an increase in weight.

(4) Chloride increase of the blood occurred simultaneously with decreased gastric chlorides as well as lowered free and total acidity of the gastric fluid.

(5) Studies of the serum pH of dogs revealed no relationship between edema formation and acidosis.

(6) The primary factor for edema formation is thought to be the lowering of the colloidal osmotic pressure of the plasma due to a low proteinemia.

I wish to thank Dr. S. Morgulis, Professor of Biochemistry of the University of Nebraska College of Medicine whose aid and suggestions made this study possible.

REFERENCES

(1) Achard, C., Ribot, A., and Leblanc, A.: Le coefficient lipémique dans les hydropisies. R. C. Soc. Biol., **82:** 339–344. 1919.

(2) Barker, M. H., and Kirk, E. J.: Experimental edema (nephrosis) in dogs in relation to edema of renal origin in patients. Arch. Int. Med., **45:** 319–346. 1930.

(3) Cullen, G. E., and Bülmann, E.: The use of the quinhydrone electrode for hydrion concentration determination on serum. Jour. Biol. Chem., **64:** 727–738. 1925.

(4) Cullen, G. E., and Earle, I. P.: On the determination of the pH of the blood. Jour. Biol. Chem., **76:** 565–581. 1928.

(5) Elwyn, Herman: Edema and its treatment. New York: MacMillan Company, 1929, pp. 182.

(6) Fischer, M. H.: Oedema: A study of the physiology and the pathology of water absorption by the living organism. New York: J. Wiley & Sons, 1910, pp. 209.

(7) Gilman, A., and Cowgill, G. R.: Osmotic relations of blood and glandular secretions; regulatory action of total blood electrolytes on concentration of gastric chlorides Am. Jour. Physiol., **99:** 172–178. 1931.

(8) Hawk, P. B., and Bergeim, O.: Practical physiological chemistry, Ed. 9. Philadelphia. P. Blakiston's Son Co., 1926, pp. 931.

(9) MacLean, H., and Griffiths, W. J.: Factors influencing concentration of hydrochloric acid during gastric digestion. Jour. Physiol., **65:** 63–76. 1928.

(10) Morgulis, S., and Hamsa, W. R.: Studies on urinary acidity and a method for electrometric titration of urine. Jour. Biol. Chem., **74:** 851–861. 1927.

THE MECHANISM OF JAUNDICE: A WORKING HYPOTHESIS*

NORMAN W. ELTON

St. Joseph's Hospital, Reading, Pa.

In the following discussion jaundice is considered a qualitative or quantitative abnormality in serum bilirubin, detectable by the determination of the icterus index, van den Bergh reaction, and bilirubin content of serum. Visible jaundice implies sufficient yellowish discoloration of sclera, skin, or mucous membrane to be detectable by simple observation alone. Jaundice is frequently not visible, and reliance on visual acuity results in failure to detect its existence in the majority of cases.

The threshold of visibility is a decided variable and cannot be postulated in terms of the icterus index or quantitative bilirubin. A clear skin and pearly white scleras may be noted in a white patient dying of carbon monoxide poisoning when the icterus index is 25, the bilirubin 2.3 mgm./100 cc., and the van den Bergh reaction positive; and on the other hand, an intense skin jaundice may develop in a white patient with lobar pneumonia when the icterus index rises only from 6 to 10 and the bilirubin from 0.4 to 0.6 mgm./100 cc., also with a positive van den Bergh reaction. Many similar experiences could be mentioned. Intense icterus in the negro frequently is not observed at all. The visibility of jaundice means little without accurate knowledge of its duration, character, and fluctuations. Such data are available only through the serial application of the bilirubin tests in a given case.

The fundamental bases of the working hypothesis presented herewith as an explanation of the mechanism of jaundice are the van den Bergh reaction and the division of the liver unit, the lobule about its central vein, into functional zones from the standpoint of bilirubin excretion.

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

THE VAN DEN BERGH REACTION

The term van den Bergh reaction is advisedly employed in a specific sense with reference only to the qualitative procedure. Hijmans van den Bergh, while working on the adaptation of Ehrlich's diazo reaction to the detection and measurement of bilirubin in body fluids, as recommended by Pröscher, one day made an accidental discovery. A bile specimen to which it had been forgotten to add alcohol, as required by the technic of Ehrlich, nevertheless yielded an immediate color change when the diazo reagent was added. This mistake and timely observation promptly led to the recognition of two forms of bilirubin in blood serum: one, water-soluble, reacting readily with the diazonium bodies in a purely aqueous medium; the other, insoluble in water, requiring the addition of alcohol to provide a common solvent for itself and the diazonium bodies. The composite findings of van den Bergh,[2] Blankenhorn,[3] Collinson and Fowweather,[4] M'Gowan,[11] and others make it quite clear that the water-soluble, or crystalloid form, is a bilirubinate derived from the acidic pigment, bilirubin, when the latter undergoes neutralization by an alkali, and that the second form is bilirubin in its original state.

When bilirubin is added to a neutral or acid aqueous solvent, it will not dissolve, but remains suspended in a particulate state. If alkali is added, drop by drop, to this bilirubin suspension, the bilirubin particles diminish in size and a faint yellowish tint is gradually imparted to the medium in which the particles are suspended. This yellowish color remains faint until, on the addition of a final drop of alkali, the bilirubin suddenly and practically completely dissolves, and the solution becomes deeply colored and clear. This occurs at the iso-electric point for bilirubin, which apparently lies in the neighborhood of pH 11.0. If this bilirubinate solution is gradually acidified again, the iso-electric point cannot be accurately identified by the precipitation of bilirubin, because, due to the operation of an induction period, the precipitation is slow and delayed, and a relatively high degree of acid change may be attained before the phenomenon can be

observed. In albuminous fluids, such as serum, the proteins will adsorb bilirubinate, will act as protective colloids, and further retard precipitation. The phenomenon of the induction period alone is sufficient to explain why bilirubinate will react with the diazonium bodies in the mineral acid medium of the diazo reagent. Van den Bergh[2] originally observed that the addition of bile salt to a carefully prepared bilirubin suspension yielded sufficient bilirubinate to cause an immediate diazo reaction without alcohol. This is a very significant observation, for it indicates the manner in which bilirubinate may be formed from bilirubin in the polygonal cells of the liver where bile salt is produced.

TECHNIC OF THE REACTION

A cubic centimeter of clear serum is placed in a 15 cc. graduated centrifuge tube* having a tapering lower end. The tube is slanted to an almost horizontal position and 0.5 cc. of Ehrlich's diazo reagent is allowed to run down the inside of the tube from a pipette so that it will overlay the serum. The tube is then restored to the vertical position and the contact zone of the two fluids is examined for the development of a reddish ring. Should no color change appear within about 60 seconds, the tube is then gently shaken to lower the contact zone and to mix a portion of the serum with the reagent. Another contact zone will form nearer to the bottom of the tube, and the undisturbed serum below this zone may be compared in color with the overlying serum-reagent mixture. This technic affords a color control in the same tube in which the reaction is carried out, allowing color changes and their speed readily to be observed. This technic frequently makes it possible to detect positive reactions when by the original procedure the serum would have yielded a delayed or even a negative reaction. Reactions with delay periods of less than 10 minutes when this technic is used are definitely associated with the positive reaction itself. The speed of the reaction in attaining full color density after the first color change is detected appears of no demonstrable clinical significance. It is primarily a test for the presence or absence of bilirubinate that reacts within 60 seconds.

Positive van den Bergh reactions may occur in serums with any icterus index or bilirubin content. They have been observed, in a series of over 2000 serums, from the icterus index 1.2 to the icterus index 360. In the new-born the chance of obtaining a

* Amounts are specified because of the quantitative bilirubin determination which follows upon the determination of the van den Bergh reaction.

positive reaction on fontanelle blood during the second to tenth day of life is 36 per cent, and on the sixth day attains the peak of 55 per cent.[7] During the course of the icterus resulting from a traumatic interstitial blood extravasation, undeniably icterus with a hemolytic origin and of the overload type, positive reactions are often obtained.[6] Obviously the findings resulting from the application of the ring test modification of the van den Bergh reaction compel some readjustments in prevailing concepts of the nature of jaundice.

This ring test modification was originally introduced because of recalling the ring test technic used in conducting the diazo reaction on urine, in which an ammonia solution is overlayed on the urine-reagent mixture. Recently Magath[13] has pointed out that Lepehne[10] described an identical ring test technic for the van den Bergh reaction in 1921. Lepehne, however, after a few trials, failed to appreciate its value, for he came to the conclusion that this modification afforded no advantage over the original technic in dealing with serums of low bilirubin content and discontinued its use.

INADEQUACY OF PREVAILING THEORIES OF JAUNDICE, AS BASED ON THE VAN DEN BERGH REACTION

It is now generally realized that intrahepatic jaundice cannot be differentiated from the true obstructive form by means of the van den Bergh reaction alone. Perhaps the greatest fallacy in prevailing theories of jaundice lies in the acceptance of the assumption that jaundice associated with a negative van den Bergh reaction is of hemolytic origin and a manifestation of excessive blood destruction with a consequent overproduction of bilirubin. This phenomenon has been explained by assuming that bilirubin is transported to the liver sinusoids in such large amounts that it cannot all be handled by the liver, and much of it passes through into the central veins unchanged.[15] If this assumption were true, then with each circuit of the blood through the liver an additional increment of bilirubin must be added constantly to the steady overproduction from bone marrow and spleen, until, in a short time, the patient should become saturated with biliru-

bin. Yet these patients usually exhibit only minor fluctuations in their serum bilirubin levels, and a definite tendency to a rather constant level. Furthermore, studies on the icterus resulting from known overloading of normal livers, resulting from the release of bilirubin from interstitial blood extravasations,[6] show that, contrary to existing beliefs, a positive van den Bergh reaction characterizes the icterus of the overloaded normal liver. This observation meets with no small degree of confirmation in the recent finding of Mann and Bollman[15] that a positive van den Bergh reaction develops following hemoglobin injection in mammals. Such findings appear quite decisively to refute the persistent negative van den Bergh reaction as a criterion of hemolytic jaundice.

Familial jaundice, characterized by the continued exhibition over a long period of time of a negative van den Bergh reaction, rarely accompanied by any symptomatic disturbance, is demonstrable in from 2 per cent to 3 per cent of the white race. Van den Bergh aptly describes this condition as physiological hyperbilirubinemia, analogous to the cholémie simple familiale of the French clinician Gilbert,[9] and states that it seems more prevalent in Jews. Since the van den Bergh reaction remains negative, and the bilirubin level changes very little during the long periods of quiescence in this condition, no increased blood destruction can possibly be postulated (except perhaps during exacerbations, when the van den Bergh reaction may become positive), and the high bilirubin level can rationally be interpreted only as indicative of an elevated excretion threshold, as exists normally in the horse.

Another type of icterus which has defied explanation is the rapidly rising and rapidly subsiding jaundice of great intensity accompanied by a positive van den Bergh reaction throughout its course, and in which no duct obstruction or liver damage can be demonstrated. The following daily changes in serum bilirubin content illustrate an extreme form of such a condition: 5.3, 7.0, 8.0, 13.5, 5.0, 5.4, 2.9, 1.9, and on down. It is obvious that if liver damage were the primary factor in the production of such icterus, then damage sufficient to elevate the serum bilirubin

level to 13.5 mgm/100 cc. could not be so completely repaired in five days. This type of jaundice appears to be functional in origin, and will be analyzed further.

THE BILIRUBIN EXCRETION MECHANISM

Mann[17] has shown that jaundice develops rapidly in a mammal (dog) following single stage hepatectomy, and that the bilirubin in the blood in this jaundice is negative in terms of the van den Bergh reaction. This indicates not only that bilirubin is chiefly of extrahepatic origin in a mammal, but also that the action of the liver parenchyma is involved in the conversion of bilirubin into its positive reacting salt, or bilirubinate. So far as is known, the dog kidney alone holds the unique position of being the only mammalian organ other than the liver which can do this.[17] Bilirubin, however, is normally produced in the bone marrow and spleen, and also at sites of interstitial hemorrhage and from blood extravasated into serous cavities, and, since it is not water-soluble, must enter the blood from its various sites of origin as a suspensoid colloid. Sribhishaj, Hawkins and Whipple[16] estimate that 1 gram of hemoglobin yields 40 milligrams of bilirubin when it breaks down. Hence it may readily be understood why a relatively small blood extravasation may contribute an appreciable surplus of bilirubin, rapidly absorbable from interstitial foci, although resisting absorption from serous cavities because of its colloidal state.

The physiology of excretion, as well as of absorption, implies a mechanism of solubility. Hence the liver, in excreting bilirubin, must first convert it to a bilirubinate. Bile salt, alkaline in reaction, is elaborated in the liver parenchyma, and provides an ideal medium in which the acidic pigment, bilirubin, may be converted by neutralization to a bilirubinate.

Geraudel[8] conceived an ingenious theory of the bile secretion mechanism. In the light of the knowledge of that time, he believed that bile pigment was elaborated entirely in the liver along with the other bile constituents, and as a result of red cell destruction in the peripheral zone polygonal cells of the liver lobules. He visualized an intense local cholemia in the sinusoids, reduced

by the removal of bile pigment from the sinusoidal blood in the central zones of the liver lobules. The mere substitution of bilirubin for the red cell in the context of his theory makes his logic strikingly significant at the present time. The following is an excerpt from his paper:

"When the duct of any gland is obliterated, the gland atrophies. When the bile ducts are obliterated, the central zone cells alone in the liver lobule undergo atrophy, while the portal (peripheral) zone cells remain unchanged. Atrophy thus indicates the cells involved in the excretion process Two functionally distinct zones exist: (1) the portal (peripheral) zone, concerned with internal secretion; (2) the central zone, concerned with external secretion. The peripheral zone polygonal cells present inert walls to the bile canaliculi. The injection of bile into the portal blood of animals shows that when an overload of pigment passes through the liver, the central zone cells alone exhibit pigment congestion. The normal balance between these two zones appears difficult to maintain. Damage to the central zone cells is an important factor in the etiology of jaundice."

A cross section of a liver lobule suggests a spoked wheel, with the substitution of the central vein for the hub, and with anastomosing branches on the spokes, each branch equal in thickness to the original thickness of the spokes. Sinusoids occupy the spaces. The spokes are straight only for a short distance from the central vein. Since the afferent blood supply is from the periphery, the sinusoidal stream bed undergoes marked narrowing as it converges on the central vein, resulting in a pooling of the sinusoidal blood in the periphery of the lobule and a great acceleration in the rate of flow in the central zone in order to maintain the minute volume flow through the lobule as a whole. The pooling of the blood in the peripheral zone will facilitate interchanges between blood and liver elements, while the rapid flow in the central zone will tend to minimize such interchanges. It will be, then, in the peripheral zone where the Kupffer cells, with their tendril-like pseudopods and known affinity for colloids, can most readily phagocytize bilirubin particles and pass them on into the underlying liver parenchyma for conversion and excretion.*

* The writer has noted photomicrographs of normal human livers after thorotrast injection in which the Kupffer cells of the peripheral zones were heavily laden with thorium dioxid, while those of the central zones were but slightly or not at all involved.

Another implication of the normal anatomical design of the liver lobule becomes evident when it is appreciated that the pigment load is relatively greatest in the central zone cells, for each of the end pieces of the liver cords about the central vein is fed by at least six cords of equal cell volume from the peripheral

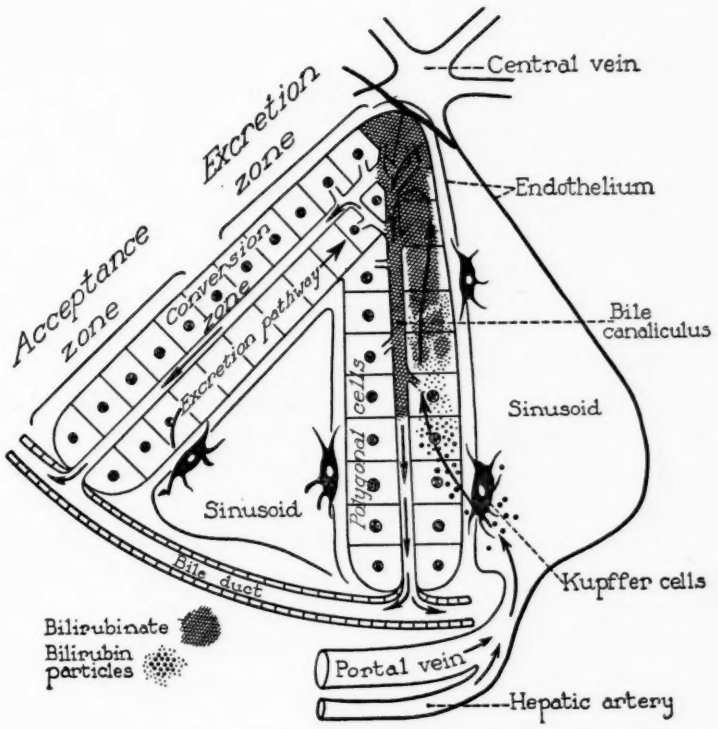

FIG. 1. BILIRUBIN EXCRETION MECHANISM

Radial structure of liver lobule causes marked narrowing of sinusoidal stream-bed as it converges toward the central vein and a marked reduction in number of polygonal cells carrying original pigment burden from peripheral parenchymal afferents.

parenchymal afferents. Whereas the peripheral parenchyma may readily take care of a pigment burden, this burden will be increased six-fold for the central zone cells. This consideration seems to explain the increased sensitivity of the central zone cells to pigment overloads, and also to parenchymal damage in view of the additional factor of their relatively less efficient

blood supply in an organ already normally functioning under conditions of partial anoxemia.

The excretion pathway from Kupffer cell to bile canaliculus through the lobular zones is clearly outlined in retrograde by the microscopic study of bile retention in liver lobules when jaundice is present and its intensity has been determined on the day of death. In low grade jaundice bile retention is seen involving only the polygonal cells about the central vein. As the degree of jaundice increases, this polygonal cell pigmentation radiates in the cords toward the periphery of the lobule, until, in the most intense grades, the Kupffer cells also become pigment-laden. Thus the excretion pathway may be traced backward to the point of entrance of the pigment into the liver parenchyma.

The liver lobule, from the standpoint of bilirubin excretion, may be divided into two major functional zones and an intermediate zone. These are: (1) the *acceptance zone* (peripheral portion), where bilirubin is accepted by Kupffer cells and passed on into the underlying liver parenchyma for conversion and excretion; (2) an *intermediate zone* between the peripheral and central regions, where bilirubin is converted by neutralization to bilirubinate, thereby becoming water-soluble; (3) the *excretion zone* (central), where bilirubinate enters the bile canaliculi from the polygonal cells, and through which it passes as a bile constituent to the periphery and into the ducts.

It is likely that these zones are not necessarily sharply demarcated, and that in stress any one zone may assume to some degree the functions of the others.

Figure 1, a diagram of the liver lobule, graphically illustrates this mechanism.

FACTORS INVOLVED IN THE ETIOLOGY OF JAUNDICE

1. Mechanical obstruction of the bile ducts

This factor is so well known that it requires little discussion. The visible jaundice which it causes is rarely overlooked. When bilirubinate regurgitates into the blood stream by way of the liver lymphatics and other channels, the van den Bergh reaction will obviously be positive.

2. Dynamic elevation of the excretion threshold: existence of a reactive state in the liver

The rapidly ascending and descending jaundice of marked intensity associated with the development of a positive van den Bergh reaction, may be understood on the following basis. It is assumed that the liver, like other organs, conforms to the principle of the "all-or-none law." A liver unit, the lobule, either excretes the bilirubin at its full capacity in its sinusoids or rests and allows it to pass through. Lobules alternate in phases of rest and activity. A certain (probably very small) proportion of the total number of liver lobules must always be in action to maintain the normal low blood bilirubin level. If, due to the operation of an icterogenic factor, a greater number of lobules are withdrawn from action than is compatible with the maintenance of the normal blood bilirubin level, not only will the pigment increase in the blood, but also the functioning lobules will soon become overloaded. Overloading will result in the leakage of bilirubinate from the congested central zone cells and the concentration of bilirubinate, as well as of bilirubin, will rise in the blood. Since lobules will continue to alternate in phases of activity and rest, such a liver will show microscopically a fairly uniform bile retention in all lobules. Under this condition the liver may be said to be in a reactive state. When the icterogenic factor ceases to operate and the reactive state terminates, the liver can then rapidly excrete the retained pigment by throwing into action an adequate number of lobules. Should the reactive state terminate quickly, the jaundice will subside rapidly: should the reactive state terminate slowly, the subsidence of the jaundice will be more gradual.

This is believed to explain the nature of the jaundice in occasional cases of lobar pneumonia, apparently in catarrhal jaundice, Weil's disease, and possibly in some other entities. Liver damage is at times prone to intervene during the existence of a reactive state. A fatality in a case clinically manifesting the syndrome of catarrhal jaundice invariably causes a change in diagnosis to subacute cirrhosis or acute yellow atrophy at nec-

ropsy. The pathology of the liver in Weil's disease varies from simple bile retention in the central zones to the picture of acute yellow atrophy.

Jaundice of this nature seems to be associated with diseases in which the etiological organism is bile-soluble, such as the pneumococcus and the leptospira icterohemorrhagica.

3. Adynamic elevation of the excretion threshold

The horse is the only domestic animal found by van den Bergh constantly to exhibit a high excretion threshold for bilirubin, ranging from 0.6 to 2.6 mgm./100 cc. Occasional human individuals manifest high thresholds by maintaining levels well above the human normal of 0.2 mgm./100 cc. or less for long intervals. These are the not uncommon cases of simple familial icterus. The explanation of this apparent threshold elevation is conjectural, but nevertheless must constantly be considered in many clinical problems. Plausible explanations include: (1) a generalized decrease in the permeability of the Kupffer cells to bilirubin, whereby a higher blood bilirubin level is required to activate the excretion mechanism; and (2) an abnormality in the size of bilirubin particles to be phagocyted by the Kupffer cells. Inference derived[5] from the correlations of the icterus index, van den Bergh reaction, and quantitative bilirubin already implies that the liver is less permeable to large bilirubin particles than to small ones.

It has been pointed out in a preceding section that a negative van den Bergh reaction is in itself by no means indicative of excessive bilirubin production or blood destruction, for when a normal liver is overloaded with pigment quite the opposite obtains in that a positive van den Bergh reaction tends to develop. Hence the question of the adynamic elevation of the excretion threshold merits serious consideration in conditions manifesting jaundice of the so called hemolytic type.

4. Pigment overload

This factor has already been discussed quite at length. The sensitive central zone polygonal cells, congested by a pigment

overload, apparently readily leak their bilirubinate into the blood stream by way of the liver lymphatics, even with relatively slight overloading, resulting in the development of positive van den Bergh reactions in the jaundice that follows, even though the pigment content of the blood rises only very slightly at times.

5. Damage to liver parenchyma

Damage to the central zone polygonal cells, of toxic, infectious, or mechanical nature, if it involves the liver diffusely, impairs the efficiency of the excretion mechanism. Many diseases with which jaundice is associated manifest such parenchymal liver damage. The central zone polygonal cells will always be involved functionally or pathologically whenever a positive van den Bergh reaction develops in the peripheral blood stream.

The central zone damage due to vascular stasis in congestive heart failure of primary cardiac origin seems invariably to give rise to a positive van den Bergh reaction, often without visible jaundice, in attacks of marked severity.

6. Patent ductus venosus

During the first few days of life the portal circulation is shunted from the liver sinusoids by the patency of the ductus venosus of Arantius, which carries the portal flow directly into the vena cava. Mann has aptly remarked that the ductus venosus forms a natural Eck fistula. Recent studies on icterus neonatorum[7] question the importance of any hemolytic factor in this condition. Whatever other factors may be operative in icterus neonatorum, the patency of the ductus venosus must assume a position of major significance.

The listing of the above factors believed to be operative in the etiology of jaundice is not intended to imply that only one may exist at any given time. Two or more may often coexist.

Experience in a rather exhaustive study of the mechanism of jaundice and the behavior of jaundice in a number of clinical entities has led to the conviction that any classification of jaundice is not only difficult and clinically impracticable, but must inevitably breed fallacies if attempted. It is safe only to assert

that jaundice is the result of bilirubin retention, bilirubinate regurgitation, or both phenomena occurring simultaneously.

The recent phraseology of Ludwig Aschoff well expresses the situation: "Ohne mangelhafte Ausscheidung des Gallenfarbstoffs durch die Leber, kein Ikterus." Thus the somewhat ambiguous dictum of Naunyn and Minkowski, "Ohne Leber, kein Ikterus," becomes clarified when interpreted as, "without (involvement of) the liver, jaundice cannot occur."

SUMMARY

Application of the ring test modification of the van den Bergh reaction to the study of jaundice reveals many data inconsistent with prevailing classifications and concepts of its nature. The commonly accepted concept of hemolytic jaundice has been found to be especially fallacious. With these newer data a working hypothesis has been constructed to explain the mechanism of jaundice on the basis of the bilirubin excretion mechanism, disturbances of which are operative in producing it. Data from the work of many investigators have been correlated in this analysis.

The liver is the excretory organ for bilirubin. Bilirubin in the mammal is produced chiefly extrahepatically. Being insoluble in aqueous solvents except alkalis, bilirubin enters the blood stream from its sites of origin in a state of colloidal suspension. The normal alkalinity of the blood is insufficient to change its nascent state. The liver, in excreting bilirubin, converts it into a water soluble salt, a bilirubinate. Since bilirubin is an acidic pigment, the alkaline bile salt of the liver parenchyma is believed to be the agent which neutralizes the pigment sufficiently to form the salt.

The structural design of the liver lobule, the known affinity of the Kupffer cell for colloidal particles, and a slight modification of the bile secretion mechanism as conceived by Geraudel, lead to the division of the liver lobule, from the standpoint of bilirubin excretion, into two major functional zones and an intermediate zone, as follows:

(1) *Acceptance zone*, involving the peripheral portion of the lobule, where bilirubin is taken up from the slow-flowing sinus-

oidal stream by Kupffer cells and passed on by them into the underlying polygonal cells for conversion and excretion.

(2) *Conversion zone*, an intermediate zone midway between the periphery and the center of the lobule, where bilirubin is converted to a water-soluble salt, a bilirubinate, as it passes down the liver cords through the polygonal cells toward the central portion of the lobule.

(3) *Excretion zone*, involving the central portion of the liver lobule, where bilirubinate is finally excreted as a bile constituent by the central zone polygonal cells into the bile canaliculi, through which it passes to the periphery and into the ducts.

The nature of jaundice is advisedly considered from the point of view of etiological factors rather than of classification. Etiological factors have been postulated as follows: (1) mechanical obstruction of the bile ducts; (2) dynamic elevation of the excretion threshold; (3) adynamic elevation of the excretion threshold; (4) pigment overload; (5) damage to liver parenchyma; (6) patent ductus venosus.

REFERENCES

(1) Aschoff, Ludwig: Über Gallenfarbstoffbildung und Gelbsucht. Klin. Wochenschr., **11:** 1620–1624. 1932.

(2) Bergh, A. A. Hijmans van den: Der Gallenfarbstoff im Blute, ed. 2. Leipzig: S. C. van Doesburgh, and Leiden: Johann Ambrosius Barth, 1928, pp. 29–35.

(3) Blankenhorn, M. A.: Acholurie jaundice. Arch. Int. Med., **27:** 131–134. 1921.

(4) Collinson, G. A., and Fowweather, F. S.: An explanation of the two forms of bilirubin demonstrated by the van den Bergh reaction. Brit. Med. Jour., **1:** 1081–1083. 1926.

(5) Elton, N. W.: Physiology, correlations, and technic of the van den Bergh reaction, icterus index and quantitative serum bilirubin. Jour. Lab. and Clin. Med., **17:** 1–13. 1931.

(6) Elton, N. W.: Postoperative latent jaundice. Surg., Gyn. and Obst., **53:** 656–661. 1931.

(7) Elton, N. W.: The van den Bergh reaction in icterus neonatorum. In press.

(8) Géraudel, Émile: Ictere et sécrétion biliaire. Jour., Physiol. et Path. Gen., **8:** 103–114. 1906.

(9) Gilbert, A., and Herscher, M.: Cholémie physiologique Presse Med., **26:** 201–202. 1906.

(10) LEPEHNE, G.: Weitere Untersuchungen über Gallenfarbstoff in Blutserum des Menschen. Deutsche Arch. f. Klin. Med., **135:** 79–107. 1921.

(11) M'GOWAN, J. P.: Some aspects of the van den Bergh test for bilirubin. Edin. Med. Jour., **36:** 242–248. 1929.

(12) MCNEE, J. W.: Jaundice: a review of recent work. Quart. Jour. Med., **16:** 390–420. 1923.

(13) MAGATH, T. B.: The serum bilirubin test. Jour. Lab. and Clin. Med., **18:** 974–980. 1933.

(14) MANN, F. C.: Abstract of discussion. Jour. Am. Med. Assn., **95:** 1072. 1930.

(15) MANN, F. C., AND BOLLMAN, J. L.: Jaundice: a review of experimental investigations. In press.

(16) SRIBHISHAJ, K., HAWKINS, W. B., AND WHIPPLE, G. H.: Bile pigment and hemoglobin interrelation in normal dogs. Am. Jour. Physiol., **96:** 449–462. 1931.

NEW ANTIGENS FOR THE KOLMER MODIFICATION OF THE WASSERMANN TEST

JOHN A. KOLMER

From the Research Institute of Cutaneous Medicine and the Department of Medicine of Temple University, Philadelphia, Pennsylvania

As previously reported by Kolmer and Richter[3] it is possible to increase the antigenic sensitiveness of my cholesterolized and lecithinized alcoholic extract of beef heart (designated as C. L.) by adding an excess of acetone insoluble lipoids. This increases the anticomplementary activity very slightly and does not render the extract at all hemolytic. But antigenic activity is definitely increased with no increase of falsely positive or non-specific reactions so that it is possible to use 20 antigenic units instead of the usual 10 units in conducting my modification of the Wassermann test with a corresponding increase in the specific sensitiveness of the reactions.

SOURCE OF ACETONE INSOLUBLE LIPOIDS

The necessary acetone-insoluble lipoids (lecithins) required for this additional sensitization are easily secured from the ether extractions of beef heart powder (Difco Laboratories) used in the preparation of antigen for the Kahn test.

In the preparation of the latter, 50 grams of beef heart powder are extracted with 200 cc. of ether followed by three additional extractions of 150 cc. each making a total of about 650 cc. of ether.

These ether extracts contain from 1.2 to 2.0 grams of acetone-insoluble lipoids of proved antigenic value. They are easily secured by mixing the ether extracts, evaporating to about one-fifth volume and adding six volumes of acetone. The lipoids are thereby precipitated and readily secured by decanting the acetone and fanning the residue.*

* These lipoids can be kept in acetone or as a paste until needed. Experience has shown that they are perfectly preserved either at room temperature or in a refrigerator.

My new antigen utilizes these lipoids. When 2 grams dissolved in ether are added to 100 cc. of C. L. antigen there is a resulting increase of antigenic sensitiveness with but a negligible increase of anticomplementary activity.

Unfortunately however, not all of these acetone-in-soluble lipoids go into complete solution but even when the excess is filtered off through fat-free paper the resulting clear extract maintains its increased antigenic activity. This suggests that the acetone insoluble lipoids (lecithins) contain a lipoid freely soluble in alcohol and ether, the exact identity of which I hope to determine by further investigation.

Therefore in those laboratories preparing antigens for the Kahn and Kolmer tests it is advised to recover the acetone-insoluble lipoids from the ether extracts of the Kahn antigen and use them for increasing the sensitiveness of the Kolmer antigen as follows:

METHOD OF PREPARING NEW C. L. ANTIGEN

(1) Twenty-five grams of beef heart powder (Difco Laboratories) are extracted for five days at room temperature with 200 cc. of ether and the ether saved.

(2) The residue is extracted with 200 cc. of ethyl alcohol in an incubator for four days and the alcohol saved.

(3) The latter is evaporated and the residue extracted with 30 to 50 cc. of ether. This ether is mixed with the ether of the primary extraction, concentrated and treated with six volumes of acetone to precipitate the acetone-insoluble lipoids (lecithins).

(4) After extracting the tissue residue a second time with 100 cc. of absolute ethyl alcohol in an incubator for six days these lipoids, along with 2.0 grams of additional lipoids recovered from the ether extractions of the Kahn antigen and 0.2 gram of cholesterol are dissolved in 20 cc. of ether and added to the secondary alcoholic extract.

(5) The mixture is shaken well and placed in a water bath at 55°C. for one hour to aid solution of the lipoids. After allowing to stand at room temperature for several days with daily shaking, the extract is filtered through fat-free paper to remove the undissolved lipoids, measured and sufficient absolute ethyl alcohol (acetone free) added to bring the total volume to 100 cc.

(6) The resulting clear extract is titrated in the usual manner[1, 2] except that the dilutions in the antigenic titration are carried up to about 1:10,000, since the extract has increased antigenic activity with slight or no increase of anti-complementary activity.

(7) Under the conditions *it is used in dose of 20 units* instead of 10 units for conducting the Kolmer test which increases the sensitiveness of the test without increasing the danger of falsely positive or non-specific reactions.

The antigen should be kept at room temperature where its properties remain unchanged for a year or more when tightly stoppered to prevent evaporation.

The units of C. L. antigen before re-enforcement with the additional lipoids are usually as follows:

Hemolytic unit: Absent in 0.5 cc. of 1:4
Anticomplementary unit: 0.5 cc. of 1:6
Antigenic unit: 0.5 cc. of 1:2400

After adding 2.0 grams of acetone-insoluble lipoids to 100 cc. as described above, antigenic activity is increased without any change in anticomplementary and hemolytic activity:

Hemolytic unit: Absent in 0.5 cc. of 1:4
Anticomplementary unit: 0.5 cc. of 1:6
Antigenic unit: 0.5 cc. of 1:5000 or higher

The dose of 20 units is 0.5 cc. of 1:250 or higher which is at least 40 to 60 times less than the anticomplementary unit and giving therefore a wide range of safety.

ALTERNATIVE METHOD

I have also found that the technic of preparing the antigen can be simplified and antigenic sensitiveness still further increased after one of the Noguchi methods by extracting beef heart powder with acetone; discarding the acetone and drying the residue which is then extracted with absolute ethyl alcohol and re-enforced with 0.2 grams of cholesterol and 2.0 grams of acetone-insoluble lipoids. The primary extraction with acetone appears to remove the objectionable hemolytic and most of the anticomplementary substances while leaving the highly antigenic acetone-insoluble lipoids which are alcohol soluble. In this manner the principles of preparation are essentially similar to the former method, the technic of preparation being as follows:

(1) Place 30 grams of beef heart powder (Difco Laboratories) in a flask with 100 cc. of chemically pure acetone. Stopper tightly; shake thoroughly and keep at room temperature for five days with brief shaking each day.

(2) Filter and discard the acetone.

(3) Dry the residue and extract with 100 cc. of chemically pure absolute ethyl alcohol in a tightly stoppered flask for five days at room temperature, shaking briefly each day.

(4) Filter through fat-free paper with slight squeezing of the tissue.

(5) Dissolve 0.2 gram of cholesterol and 2 grams of acetone-insoluble lipoids in 20 cc. of ether and add to the filtrate. Shake thoroughly and place in a water bath at 55°C. for one hour to aid solution of the lipoids.

(6) Allow to stand at room temperature for two or three days with brief shaking each day. Filter through fat-free paper. Measure and add absolute ethyl alcohol if necessary to bring the total volume to 100 cc.

(7) Titrate in the same manner as originally described[1, 2] except that the dilutions are carried up to about 1:10,000.

(8) The extract should be kept tightly stoppered at room temperature where its properties remain unchanged for a year or longer.

(9) Experience has shown that this antigen is not hemolytic; that the anticomplementary unit does not exceed 0.5 cc. of 1:10 dilution and that the antigenic unit is 0.5 cc. of 1:6000 or higher.

(10) Under the circumstances *it is also used in dose of 20 antigenic units* instead of 10 units as advised heretofore for C. L. since this dose is usually 60 to 80 times less than the anticomplementary unit and representing therefore an extremely wide and safe range.

When this new antigen is prepared by extracting beef heart powder with acetone and then with alcohol as described in steps 1, 2 and 3 above followed by the addition of 0.2 gram of cholesterol per 100 cc. the resulting extract is usually a satisfactory antigen even without re-enforcement with additional lipoids, the results being usually as follows:

Hemolytic unit: Absent in 0.5 cc. of 1:4
Anticomplementary unit: 0.5 cc. of 1:8
Antigenic unit: 0.5 cc. of 1:4000

But when re-enforced with 2.0 grams of acetone insoluble lipoids per 100 cc. as described above the antigenic activity is still further increased without any increase of anticomplementary or hemolytic activity:

Hemolytic unit: Absent in 0.5 cc. of 1:4
Anticomplementary unit: 0.5 cc. of 1:8
Antigenic unit: 0.5 cc. of 1:6000 or higher

As a general rule antigen prepared by this second or alternative method is superior to C. L. antigen re-enforced with acetone insoluble lipoids and is, therefore, to be preferred; furthermore it is more quickly and simply prepared.

CONCLUSIONS

(1) The acetone insoluble lipoids recovered from the ether extractions of beef heart powder (Difco Laboratories) in the preparation of Kahn antigen have definite antigenic value.

(2) The addition of 2.0 grams of these lipoids to the Kolmer cholesterolized and lecithinized alcoholic extract of heart (C. L. antigen) definitely increases its antigenic sensitiveness with slight or no increase in anticomplementary activity.

(3) A simplified or alternative method of preparing this antigen re-enforced with 2.0 grams of lipoids per 100 cc. is described.

(4) This simplified antigen is preferred because more antigenic and simpler and easier of preparation.

(5) Because of higher antigenic sensitiveness it is recommended to use 20 antigenic units instead of 10 units of either antigen in the conduct of the Kolmer modification of the Wassermann test.

(6) These antigens in dose of 20 antigenic units increases the sensitiveness of the Kolmer complement fixation test for syphilis while preserving its higher specificity or freedom from falsely positive reactions when the test is conducted exactly as originally described.

REFERENCES

(1) KOLMER, J. A.: Serum diagnosis by complement fixation. Philadelphia: Lea and Febiger, p. 397, 1928.

(2) KOLMER, J. A., AND BOERNER, F.: Approved laboratory technic. New York: Appleton and Co., p. 463. 1931.

(3) KOLMER, J. A., AND RICHTER, C. E.: A note on acetone insoluble lipoids in relation to antigen for the Wassermann reaction. Am. Jour. Clin. Path., **4**: 235–238. 1934.

AN ANATOMICAL STUDY OF A THORACOPAGUS MONSTER DELIVERED DEAD AT FULL TERM*

L. W. LARSON AND P. W. FREISE

From the Departments of Clinical Pathology and Obstetrics and Gynecology, Quain and Ramstad Clinic, Bismarck, North Dakota

Double monsters are comparatively rare and the following case report has numerous interesting features. The etiology of the various forms of terata is unknown. The entrance of two spermatozoa into one ovum is apparently not the cause, because such fertilized ova usually die. There are two theories advanced, either of which appears possible. The monstrosity could either be due to a splitting of one primitive streak, or to the fusion of two primarily developed ova. DeLee,[1] in his discussion of the etiology of this condition, adds that "probably fission occurs." Experimentally, it can be shown that double monsters can result from one ovum and that they can develop from one germinal vesicle. The blastula stage can be separated into individual blastomeres by mechanical or chemical means and separate embryos will develop. If two embryonal areas appear, homologous twins result. If the embryonal areas are not entirely separated, united twins are produced. All gradations of the individual embryos are seen, from equality in the size of both individuals down to a normal development of one with a stunting of the other into a parasitic stage.

Possibly the best classification of double monsters is that of Foerster[2] which is as follows:

A. Terata Anadidyma—in which the fission or doubling is from the head downward. The most developed specimens show one pelvis and two legs, the trunks being separate.

---

* Read before the Thirteenth Annual Convention of the American Society of Clinical Pathologists, Cleveland, Ohio, June 8 to 11, 1934.

B. Terata Katadidyma—in which the splitting is from below upward. All grades are observed to the last where the two complete bodies are attached at the head.

C. Terata Kata-anadidyma—the fission being from both above and below.

ANATOMIC DESCRIPTION

The twins (fig. 1) were full term and of almost equal size. They weighed a total of 5.79 kgm. They were joined by a cartilaginous and soft tissue union extending from the upper portion of each sternum to a common umbilicus, the twins facing one another. The right ribs of one twin were joined to the left ribs of the other twin by a cartilaginous sternum, while the left ribs of the former were joined in a similar manner to the right ribs of the latter by a similar sternum. The heads, upper and lower extremities, and hips were separate and normal. Both twins were males, their external genitalia being normal. The single umbilical cord entered at the lower end of the fusion. The x-rays (fig. 2) show that the vertebral column in each twin is complete.

An incision was made on one side from the upper portion of the fusion to the lower portion. The bony plate, consisting of the upper ten ribs from each infant fused by a cartilaginous sternum, was removed. A single thoracic cavity separated from a common peritoneal cavity by means of a single diaphragm was found. There was a single pericardial sac and a fused heart from which two complete systems of aortic and pulmonary vessels emerged. The fused heart contained four chambers for each infant. There were two separate sets of lungs. Each infant had its own respiratory tract and esophagus. The livers were fused and there were two separate gall bladders, spleens and pancreases. The gastrointestinal tract of each infant was separate and complete throughout. Each had its own complete genito-urinary system.

DISCUSSION

Double monsters have never been diagnosed before the onset of labor. At best, twins have been suspected.

Sternopagi and thoracopagi constitute the most common forms of double monsters, there being 220 such cases reported in the

literature up to 1921 (Heil[3]). Meckel[3] found sixty females out of eighty incidences of sternopagi.

In the cases reported in the literature, a common thoracic cavity has usually been separated from a common peritoneal cavity by means of a single diaphragm. As a rule there have been two sets of lungs present with a fused heart. Various abnormalities of the chambers of the heart and its vessels have been reported. It is interesting to note that our case showed a fusion of the hearts with complete development of a set of auricles and ventricles, aorta and pulmonary vessels, for each infant. Apparently, a fused liver has been the usual finding. At times the gall bladders have been entirely absent. Various abnormalities of the gastro-intestinal tract have been found. Commonly, a separate esophagus, stomach and upper intestinal tract has been reported, with a fusion of the jejunums, and then a separation of the upper ileum into two separate portions of small intestines which merge into a large intestine for each infant. All cases reported seem to show a complete skeletal development for each twin with the exception of the abnormality at the point of union in the chest on either side of the monster.

A complete résumé of the clinical aspects of our case will be published later.

REFERENCES

(1) DeLee, J. B.: The principles and practice of obstetrics. 6th Ed. Philadelphia: W. B. Saunders Co., pp. 590–594, 1933.

(2) Foerster: Quoted by DeLee.

(3) Shaw, C. C., Brumbaugh, B. B., and Novey, M. A.: An anatomical and clinical study of a thoracopagus monster delivered alive at full term. Am. Jour. Obst. & Gynec., **27:** 655–667. 1934.

Fig. 1. Gross Appearance of the Monster

Note that fusion is present from the upper part of the sternum to the point of entrance of the single umbilicus.

Fig. 2. Roentgenogram

Note complete skeletal system of each twin with the exception of the fusion in the chest. The shadows extending down from the mouths are due to barium solution which had been injected into the gastro-intestinal tract of each infant. Unfortunately, the plates taken before this was done have been lost.

FIG. 2

FIG. 1

SURVEY OF TRAINING COURSES FOR LABORATORY TECHNICIANS IN GENERAL HOSPITALS*

KANO IKEDA

From the Pathological Laboratory of the Chas. T. Miller Hospital, Inc., and the Amhurst H. Wilder Dispensary, St. Paul, Minnesota

The training of laboratory technicians is without doubt most effectively undertaken, as a course in medical technology, by well recognized colleges and universities. To-day, however, far too few institutions of higher learning are offering such a course to meet the existing needs while the number of those who contemplate taking up this work is steadily multiplying and the demand for qualified technicians is on the increase. The situation has not only created a challenge to but placed the burden of responsibility on the clinical pathologists. Thus, it appears to be the obligation of clinical pathologists who direct properly equipped hospital laboratories, to maintain, when circumstances permit, a course of instruction and training in clinical laboratory procedures for a limited number of qualified students according to a uniform standard which shall insure the production of only properly qualified technicians. While believing firmly that the primary function of the clinical laboratory is to render aid in clinical diagnosis and the care of the patient, the clinical pathologist, nevertheless, may well extend his rôle as a teacher by contributing toward the instruction of clinical laboratory technicians whenever opportunities at his disposal justify such an undertaking. In this manner, the clinical pathologist is afforded an unique opportunity of contributing toward the training of technicians who shall perpetuate the dignity and tradition of a new profession, created according to high ideals.

* This survey was prepared for the Board of Registry of the American Society of Clinical Pathologists. Views expressed are those of the author and not necessarily of the Board.

The present survey deals primarily with the facilities offered by the clinical laboratories of general hospitals for the instruction and training of laboratory technicians. The commercial schools and private laboratories offering a short course in laboratory methods are excluded from this survey as are the ten institutions of higher rank which maintain a regular course in medical technology and the hospitals in affiliation with them.

Approximately 1000 general hospitals of more than 100 bed capacity were circularized with a questionnaire. About 400 replies were received. One hundred and thirty-six of these maintain a training course for technicians and furnished the data sought in the questionnaire. In addition, there were about thirty-three laboratories which claimed to train one or two technicians from time to time or "for their own use" for varying periods, but did not supply any data as to the manner of instruction, and three others which admitted only the fourth year students in medical technology of their local university. These were excluded from this study. Excluded from this survey because they failed to return the questionnaire, are twenty hospital laboratories which are known to maintain a course of training for technicians and two others which admit only the fourth year university students in medical technology. It is difficult to estimate the probable number of laboratories maintaining a technicians course among the remaining six hundred hospitals not heard from. However, from the study of the returns which include most of the larger and better known hospitals of the country, I am inclined to believe that there are probably not more than fifty additional hospitals which maintain such a course. An analysis of the data gathered from these 136 hospitals laboratories which form the basis of the present report, may furnish a fairly accurate picture of the present attempt on the part of the clinical pathologists to instruct and train their technical assistants, commonly known as laboratory technicians.

ANALYSIS OF DATA ON 136 HOSPITALS

Type of hospitals

The names of the hospitals were taken from the list published in the 1933 hospital number of the Journal of the American Medi-

cal Association.[1] All general hospitals, having a capacity of 100 beds or more were included. No note was made as to whether they were public or private, teaching or non-teaching, and approved for interneship or not. Of the 136 hospitals conducting a training course in their clinical laboratories, 119, or 87 per cent,

TABLE 1*

TYPES OF HOSPITALS

TYPES OF HOSPITALS	4C	2C	1C	HS	TOTAL
University hospitals	3	4	7	2	16†
Public hospitals	2	3	5	3	13‡
General hospitals (approved)	19	8	29	35	91§
General hospitals (not approved)	1	4	2	9	16
Total	25	19	43	49	136

* In all the tables 4C means entrance requirement of four years of college work; 2C, two years of college work; 1C, one year of college work; HS, high school graduation.

† Eight of these are tax supported hospitals.

‡ Five of these are teaching hospitals.

§ One of these is approved for residency only.

TABLE 2

HOSPITAL CAPACITY

BEDS	4C	2C	1C	HS	TOTAL	
100–199	5	7	13	18	43	65.4%
200–299	11	6	12	17	46	
300–399	3		5	8	16	34.6%
400–499	2	3	6	3	14	
500–1000	3	3	6	3	15	
1000 and over	1		1		2	
Total	25	19	43	49	136	

are approved for general interneship and twenty-nine, or 21 per cent, are either affiliated with medical schools as teaching hospitals or classed as public or charity institutions, or both, where opportunities for instruction are ideal (table 1). Forty-three have a capacity of less than 200 beds; forty-six, more than 200 and less than 300 beds (table 2). Eighty-nine, or 65 per cent, of the total, therefore, have a bed capacity of between one and

three hundred. Daily occupancy of these hospitals may be estimated to be, on the average, from 50 to 55 per cent of the full capacity. It is pertinent to inquire if the clinical laboratory of a hospital with a daily average of fifty patients or less can effectively conduct a course of training for technicians and it is surprising to note that only forty-seven, or 34 per cent of the total are hospitals of more than 300 beds. It is the hospitals of this latter class which best afford the opportunities for all-around training of technicians. More of this class of hospital should be encouraged to inaugurate a systematic course of training for technicians according to the program outlined by the Board.

TABLE 3

GEOGRAPHIC DISTRIBUTION

SECTION	4C	2C	1C	HS	TOTAL	
						per cent
East	15	2	10	23	50	36.8
Midwest	3	9	16	15	43	31.6
South	3	6	11	9	29	21.3
West	4	2	6	2	14	10.3
Total	25	19	43	49	136	100.0

Geographic distribution

Geographically, the 136 hospitals are distributed as follows: fifty, or 36.8 per cent, in the East; forty-three, or 31.6 per cent, in the Middle West; twenty-nine, or 21.3 per cent, in the South, and fourteen, or 10.3 per cent, in the West. Thirty-two states are represented. Pennsylvania with nineteen hospitals and New York with fifteen lead others; followed by Ohio, Illinois and Texas, each with nine, Missouri with eight, Massachusetts with seven, and Michigan and Minnesota, each with six hospitals (table 3).

Entrance requirements

Twenty-five, or 18.3 per cent, of the 136 laboratories require college graduation for entrance; nineteen, or 14.0 per cent, two

years college work; forty-three, or 31.6 per cent, one year college work and forty-nine, or 36.1 per cent, high school graduation. Compared with the figures obtained in the 1931 survey[2] (which included courses conducted by all other types of institutions as well as by hospitals), it is noted that college graduation is now required in 19.3 per cent of the total, against 8 per cent in 1931; two years college work, in 14.0 per cent against 8 per cent; one year college work, in 31.6 per cent against 5.5 per cent; and high

TABLE 4

LENGTH OF COURSE

MONTHS	4C	2C	1C	HS	TOTAL	
36				2	2	
32	1				1	
24	1	3	2	2	8	
20			1		1	20%
18	5	1	3	2	11	
16	1			2	3	
15		1		1	2	
12	12	9	32	28	81	60%
11		1			1	
9		1	1	1	3	
8		1	1	2	4	17%
7				1	1	
6	3	1	2	8	14	
Not stated	2	1	1		4	
Total.....	25	19	43	49	136	

school graduation, in 36.1 per cent against 64.0 per cent. Today, eighty-nine laboratories, or 63.9 per cent of the total, require one year college education or more for admission against only 21.9 per cent of all schools investigated in 1931.

Length of course

The length of the course ranges from six months or less to three years. Eighty-one, or 60 per cent, now require a period of twelve months for instruction and training. Twenty-eight, or 20 per cent, require more than twelve months; eleven of them requiring eighteen and eight, twenty-four months. Only twenty-

three, or 17.0 per cent, require less than twelve months; fourteen, or only 10 per cent, now requiring six months (table 4). Thus, it may be pointed out that the laboratories requiring a training period of twelve months rose from 37 per cent in 1931 to 60 per cent in the present survey and those requiring a six months period dropped from 26.7 per cent (of 127 courses) to only 10 per cent in the same period.

The decided upward shift in the entrance requirement and the lengthening of the training period to at least twelve months, among the large majority of these laboratories have resulted largely through the efforts of the Board of Registry of the A. S. C. P. which has required of a laboratory technician, a minimum educational pre-requisite of one year college work including chemistry and biology and a minimum training period of twelve months in a recognized hospital laboratory.

It appears safe to state that a large majority of the clinical pathologists, to-day, support the belief that a training period of less than twelve months is not sufficient and a high school diploma alone is inadequate, for a qualified laboratory technician.

Type of instruction

It is difficult to clearly conceive the manner in which the instruction of the students is being carried out by these laboratories, nor is it easy to evaluate the particular type of instruction employed in a given laboratory. Conditions and circumstances peculiar and particular to individual laboratories largely determine the program of instruction. This may be well organized, rigidly followed and conducive to best results or may be so loosely conducted as to be ineffective. Three major types of instruction with various modifications are employed.

The first, the most desirable but often scarcely suitable or practical in the average hospital laboratory, is one in which several hours are set aside daily for didactic lectures. This is supplemented by practical work under supervision. A few of the larger hospitals and some of those affiliated with a medical school adhere to this schedule without disrupting the routine program of the laboratory service proper. A few allow their students to

attend such of the lectures for the nurses as are of possible benefit to the technicians. The average clinical laboratory, however, is not so constituted as to permit too many didactic hours for their students with the staff members as teachers. The scheme works satisfactorily only in conjunction with a teaching institution. The present endeavor is to so counsel the laboratory directors as to encourage them, either (1) to accept for training only those who possess necessary college credits in laboratory sciences which obviate prolonged didactic hours while in training, or (2) to seek an affiliation with a local university or college to coöperate in the maintenance of a course in medical technology. Either of these alternative plans is believed to be the most satisfactory and, at the same time, the most practical of the means yet conceived to meet the present situation.

The second, the common and accepted mode of training, followed by many of the laboratory directors, is conducted somewhat as follows: the students are enrolled individually at stated intervals and assigned to a department for a stated period, to be transferred or advanced to a second department on completion of training in the first, et cetera. The scheme suggested in our previous survey[2] is usually followed, that is, the entire laboratory is departmentalized into, say, six units and the student assigned to each for two months under the director or technician in charge of the department whose duty it is to personally arrange didactic talks, assign lesson studies, conduct periodic quizzes and examinations as well as to demonstrate and supervise performance of tests. This method of instruction has been found practical and effective.

The third plan of "training" which is employed by a large number of hospital laboratories, particularly those employing one or two technicians and a part time pathologist, is strictly on a practice basis and usually follows no organized schedule. Some of these are the laboratories which enroll "students" in order to first extract their free service as helpers, in return for the opportunity of receiving practical instruction in routine technic. Such arrangement is practiced particularly by those clinical laboratories in which there is a shortage in personnel and the "students" are

taken in primarily to render the menial service which otherwise demands one or more additional paid employees. In a few instances, such a practice is apparently encouraged by the hospital administration. While the one being trained may assist in routine service of the laboratory as a personal assistant to the supervising technician and such assistance may be rightly claimed by the laboratory, the primary objective of the course should never be neglected or forgotten; any service rendered should be considered incidental. Certainly, no students should be allowed to render reports on their own initiative, while under training.

TABLE 5

NUMBER OF STUDENTS

STUDENT CAPACITY	4C		2C		1C		HS		TOTALS	
	Students	Laboratories	Students	Laboratories	Students	Laboratories	Students	Laboratories	Students	Laboratories
1–2	16	10	15	8	28	17	36	25	95	60
3–5	38	10	25	6	40	10	48	13	151	39
6–10	30	4	26	3	68	9	76	10	200	26
11–20			12	1	52	4			64	5
Over 20					22	1			22	1
Not stated		1		1		2		1		5
Totals.....	84	25	78	19	210	43	160	49	532	136

Students and instructors

From one to twenty-two students may be enrolled (table 5). Sixty laboratories train one or two students at a time; thirty-nine, from three to five; twenty-six, from six to ten, and six, more than ten. In all, 532 students may be trained by the 136 laboratories at one time or an average of 3.9 students per laboratory. The number of "instructors" including the directors varies from one to twenty (table 6). Thirty-one laboratories employ one or two on the staff; sixty-six, from three to five; twenty-eight from six to ten, and six, more than ten. There are 584 technical workers in the 136 laboratories or an average of 4.3 per laboratory. This makes a student to instructor ratio of 3.9:4.3. It would appear that the ratio of the student to the instructor in laboratories

other than those affiliated with a teaching institution, should be maintained approximately one to one instead of two to one, suggested in the 1931 report.

Forty-eight or 35.3 per cent of the laboratories do not employ registered technicians; thirteen made no reply. Fifty employ registered technicians and twenty-five employ one or more but not all registered technicians. In other words, seventy-five laboratories, or 55.1 per cent, of the total, employ one or more registered technicians on their staff. Several directors indicated their intentions of engaging only registered technicians in their laboratories.

TABLE 6

INSTRUCTORS

STAFF	4C		2C		1C		HS		TOTALS	
	Instructors	Laboratories	Instructors	Laboratories	Instructors	Laboratories	Instructors	Laboratories	Instructors	Laboratories
1–2	8	4	11	6	5	3	31	18	55	31
3–5	36	9	31	8	101	26	88	23	256	66
6–10	45	7	21	3	94	13	38	5	198	28
11–20	36	3	27	2	12	1			75	6
Not stated		2						3		5
Totals.....	125	25	90	19	212	43	157	49	584	136

It is of interest to note that the hospital laboratories located in California and New York City indicated that their technicians, while not registered with the Registry of the American Society of Clinical Pathologists, held a certificate from their respective State or City Board of Health. A control of the laboratory workers through legislation may be a final solution to the problem. Such legislative regulation of technicians is favored by few clinical pathologists. Many clinical pathologists are

of the opinion that statutory legislation regulating technicians would only bring about additional cumbersome machinery as a burden to the technician if not to the taxpayer, besides making it necessary for applicants to take examinations in each commonwealth where they may migrate. The Registry of the A. S. C. P., a voluntary and non-coercive agency exercises the same influence on technicians who seek qualification as the College of Surgeons does on the hospitals who are eager to earn its approval.—(Hillkowitz)

Whatever may be the individual opinion of the clinical pathologists as to the means of approach to this problem, it is clearly their prerogative and duty to define the scope and limitation of the responsibilities which may be safely delegated to their technicians. Sooner or later, the question may be raised as to whether or not the Board of Registry may accord reciprocal recognition to the holders of a certificate of proficiency from a governmental board, provided no conflict exists in the minimum requirements of these two respective agencies. Any move toward the mutual coöperation and understanding between the Board of Registry and the state or municipal boards in providing

TABLE 7

DIRECTORS

MEMBERSHIP OR LISTING	4C	2C	1C	HS	TOTALS	
						per cent
A. S. C. P. membership	12	11	33	14	70	51
"Council's" list	18	14	35	29	96	70
A. S. C. P. and "Council"	10	10	31	11	62	45.5
None	5	4	6	17	32	23.5
Totals	25	19	43	49	136	

sane regulatory measures for the laboratory technicians should be highly welcome.

Directors

Seventy (51 per cent) of the directors are members of the American Society of Clinical Pathologists while ninety-six (70 per cent) are on the Council's list of pathologists and clinical pathologists. Sixty-two (45.5 per cent) hold their membership in the Society and are also on the Council's list while thirty-two (23.5 per cent) are not identified with either bodies (table 7). Two of the directors hold no medical degree, one of which has a Ph.D. and the other had a two year college education and a nine months apprenticeship as a technician. Needless to state there are several well known pathologists and internists who do not have membership in the A. S. C. P. nor are they listed with the Council.

COLLEGE COURSE IN MEDICAL TECHNOLOGY

To the University of Minnesota probably goes the distinction of being the first to inaugurate a regular course in medical technology, leading to a degree. This was more than twelve years ago. Within recent years at least nine more institutions have been added to the list, so that there are, to-day, ten universities and colleges of recognized standing which offer a four year course of study and training for medical technicians. In addition, there are several others which offer a "post graduate" course in clinical pathology, primarily intended for graduates in medicine but allowing others to matriculate. One university offers a post graduate course leading to a Master's degree in medical technology.

Several laboratory directors have effected an affiliation with a local college or university whereby the fourth year students matriculated in the course in medical technology are required to spend the final year before graduation in the hospital laboratory for practical training and experience. A similar affiliation may be established between the hospital laboratories and junior colleges.

There are sixteen general hospitals which are intimately affiliated with medical schools and at least five more where teaching of the medical students is extensively carried out. The clinical laboratories of these hospitals afford opportunities most desirable for the instruction of student technicians.

CONCLUSIONS

The present survey has brought out the fact that the hospital laboratories are training technicians on a more systematic and effective basis than any time since the practice came into vogue. There has been substantial progress. Likewise, many laboratory directors have come to endorse the minimum standards for the training and the registration of technicians as proposed by the Board of Registry.

The survey also has pointed out important deficiencies and needs in the present system, particularly as it is practiced by the

smaller laboratories which seldom follow an organized program of instruction.

In order to maintain an effective course of training for technicians, the uniform, standardized requirements such as established by the Board of Registry should be carefully followed. It is particularly desirable that more of the directors of those hospital laboratories which enjoy adequate equipment and personnel as well as unlimited supply of specimens, may inaugurate a well planned course for technicians. Such a course may be most effectively conducted in conjunction with a college or university as one of its regular curricula.

The hospital clinical laboratory as a training center for technicians is no longer an expediency. It is an important part of a permanent program of education for the future laboratory technicians.

REFERENCES

(1) Hospital Service in the United States. Jour. Am. Med. Assn., **100:** 887–910. 1933.

(2) IKEDA, KANO: Survey of training schools for laboratory technicians. Am. Jour. Clin. Path., **1:** 467–476. 1931.

EDITORIAL

Concerning the Teaching of Clinical Pathology

While generalities are dangerous, and dogmatic statements are frequently subject to amendment, few will dispute the premise that the intelligent practice of medicine depends primarily upon the accurate recognition of the nature of the disease responsible for the symptoms; or, in other words, upon the formation of a correct diagnosis.

Nor can it be readily denied that diagnosis, as an essential first step in the management of disease depends upon the determination, first, of the particular function or functions departing from normal; second, of the nature and degree of impairment; and, third, whenever possible, upon the detection of the responsible cause and an appreciation of its mechanism.

While these basic and important principles may be thus easily and briefly outlined, their practical application to the study of disease is very often neither simple nor brief and the conscientious physician earnestly endeavoring to study thoroughly the clinical problem before him not infrequently finds his investigations developing many and diverse ramifications. The manifestations of disease are, in essence, expressions of reactions to varied stimuli, the nature and degree of which may often be determined by inferential deduction based upon the study of the phenomena produced in the patient. It is readily apparent, however, that no matter how skilled the physician may be in the art of physical examination, nor how keen his powers of observation, there are limits to the information to be thus obtained.

The presence or absence of abnormal substances in the excretions, the composition of the spinal fluid, numerical and cytological variations from the normal in the blood, the presence or absence of complement-fixing bodies in the serum, the level of the blood chemistry—these are but a few of the reactions for

the detection and determination of which specialized methods must be called upon, the methods of the clinical laboratory. While the many advances which have been made in these phases of the examination of the patient have added greatly to the understanding of the mechanism of disease and have greatly extended the armamentarium available to the physician for the study of the patient, it is neither always wisely used nor intelligently interpreted nor does experience demonstrate that the true function of the clinical laboratory is always clearly understood.

It is to the credit of the clinical pathologist that just as he was naturally among the first to emphasize the importance of these phases of the examination of the patient, so he was also among the first to decry and to combat a developing tendency to use the laboratory as a diagnostic "short-cut," to replace the thorough clinical study of the patient by an assortment of laboratory requisitions.

That this has occurred and, indeed, still occurs to a varying extent the experience of any clinical pathologist will corroborate; that it is based upon a failure to appreciate the basic, underlying principles of the situation seems obvious, for it is seen, not only in the older practitioner, but, which is somewhat surprising, in the recent product of medical education—the interne.

One does not expect of the hospital Resident technical skill in the laboratory, but it should not be unreasonable to expect of him some appreciation of how to use the laboratory in the study of his patients to the extent that he should know, first, what laboratory procedures are most likely to prove informative under a particular set of circumstances, and, second, just what information has been secured by their performance.

These are not matters of mere academic interest but have a practical and important bearing on his future practice. For it must be remembered that, whether the physician makes such examinations himself or has them made by others, the patient must submit to them and very often pay for them and often very naturally will expect some concrete result to follow.

Every young physician has somewhat of a smattering of information, and often indeed it is not much more than that, con-

cerning how to count blood or examine, after a fashion, the urine or other excretions and secretions. Very often, however, he is obviously very ill informed as to when such examinations are truly indicated nor which to select from the variety available. Quite often his selections seem based upon dogmatic and rule of thumb conceptions.

Let us take, for example, a patient whose illness of four days duration has about it something which suggests the possibility of typhoid fever. The laboratory will very probably receive a request for a leukocyte count and a Widal test, and should there be a leukocytosis, or at least, an absence of leukopenia and a negative agglutination reaction, these findings will very often be the subject of discussion.

It must be remembered that by far the greater number of internships are served, not in large, well-appointed institutions with teaching affiliations, but in hospitals of average size and average appointment whose staff, no matter how long experienced, are not all always skilled in imparting to others what they have learned. For, as everyone knows, it is one thing to possess information and very definitely another to possess also the ability to pass it on to others.

The experience of every laboratory director in the average hospital furnishes examples ad nauseum of the failure to utilize the full extent of the resources of the clinical laboratory, a failure arising very clearly from lack of clarity in the appreciation of its status and function as well as a mental haziness concerning the significance, interpretation, and clinical application of its results.

Nor is it strange, when one considers the crowded medical curriculum, that much of what is taught has to be taught in rather sketchy and dogmatic fashion, so that it is not difficult to understand how certain diseases in the mind of the student come to be associated with certain laboratory tests. For what is described to him is a typical case and what he seldom appreciates is the fact that the typical case is really a composite description of many cases and as such subject to many and sometimes marked individual variations.

While, for obvious reasons, it is apparent that the student

should have some knowledge of how to do such laboratory procedures as will later be feasible in practice, it seems equally important that he should be taught something of when they should be done and how to interpret and apply their results; or, in other words, that he should hear something of what might be called applied clinical pathology.

It is true that the medical curriculum is now overcrowded and that it is difficult to find room for new subjects. On the other hand it might be suggested, albeit with some trepidation, that many an hour is spent observing from the benches, splenectomies, shoulder-joint disarticulations and major surgical procedures in general which, perhaps, might be spent to better advantage—even in the study of applied clinical pathology, for example. After all, the recent graduate will seldom be called upon to do any major surgery in the first year or so of his practice—even if his diploma does read "Physician and Surgeon." But he will at once be faced with the necessity of examining his patient, and the very first one may deserve a thorough clinical laboratory study, not in the sense that a large number of investigations are required, but rather in that they must be intelligently chosen, carefully interpreted, and wisely applied to the solution of the clinical problem at hand. Under such circumstances the difference between the man who knows only clinical laboratory methods and the one who knows how to apply them intelligently will be evident and appreciable in his subsequent management of the case.

—R. A. KILDUFFE.

NEWS AND NOTICES

The Evaluation of Serodiagnostic Tests for Syphilis in the United States

In August, 1933, a member designated by the American Society of Clinical Pathologists, investigating the feasibility of a serologic conference for the diagnosis of syphilis in this country, approached the United States Public Health Service in order to ascertain whether assistance might not be obtained for this project. After some delay funds for this study were made available, and preliminary steps in its organization were begun in the summer of 1934. The actual work of organization was assigned to a committee of six members: two clinical pathologists, Doctors Arthur H. Sanford of Rochester, Minnesota and Walter M. Simpson of Dayton, Ohio; two syphilologists, Doctors H. H. Hazen of Washington, D. C. and Francis E. Senear of Chicago, Illinois; and two officers of the Public Health Service, Surgeon General H. S. Cumming and Passed Assistant Surgeon R. A. Vonderlehr. In selecting this committee the chief object was to enlist the services of physicians who were well qualified in their field of work. At the same time it was equally essential that they have not the slightest connection with any serologist or serologic laboratory which might participate in the evaluation study.

The program for the evaluation of serodiagnostic tests for syphilis in the United States has been completed and published in several of the medical journals of the country. In organizing this project the first aim of the committee has been to outline a procedure which duplicates, as far as possible, that of a physician in private practice who collects a blood specimen and sends it to a serologist for an examination. The committee has likewise been deeply concerned with the problem of placing comparable samples of blood in the hands of all participating serologists at approximately the same time. It is hoped that the

final results will permit a separate appraisal of the various modifications of complement fixation and flocculation tests for syphilis employed in this country. This appraisal should be based upon both the specificity and sensitivity of the several tests and, to a lesser extent, upon such factors as adaptability of the test to use by other technicians and rapidity of performance. The careful selection of donors, which has been planned, should facilitate the ultimate evaluation.

The serologists of the United States, who will participate in the evaluation study, are enumerated below:

WALTER V. BREM, Los Angeles, California.
HARRY EAGLE, Philadelphia, Pennsylvania.
WILLIAM A. HINTON, Boston, Massachusetts.
F. M. JOHNS, New Orleans, Louisiana.
REUBEN L. KAHN, Ann Arbor, Michigan. (Performing Kahn Standard Flocculation Test only.)
B. S. KLINE, Cleveland, Ohio. (Performing Kline Diagnostic Slide Test only.)
JOHN A. KOLMER, Philadelphia, Pennsylvania.
M. B. KURTZ, Lansing, Michigan. (Performing Kahn Presumptive (Quantitative) Test.)
N. H. LUFKIN, Minneapolis, Minnesota.
CHARLES R. REIN, New York, New York. (Performing Kline Exclusion Slide Test.)
E. HENRY RUEDIGER, San Diego, California.
U. S. Army Medical School, Washington, D. C.
EMIL WEISS, Chicago, Illinois.

It has been a source of regret both to the Public Health Service and to the American Society of Clinical Pathologists that many other serologists, who have requested an invitation to participate, could not be included in the evaluation study. The desire, however, to provide each participant with comparable samples made it necessary to limit the number of serologists to those who had described an original complement fixation or flocculation test which is more or less in general use.

The need for periodic evaluation of serologic work in every laboratory of the United States is thoroughly appreciated. This applies both to privately operated serologic laboratories and to

laboratories supported by public health organizations. It is hoped that some practical method may be worked out whereby the reliability of serologic procedures in any laboratory in the United States may be determined. Further potentialities also exist in the possibility of launching a series of comprehensive coöperative studies by the serologists of the country under the direction and supervision of a similar committee. Important and complex problems in the serology of syphilis, which might not be satisfactorily solved by the work of one individual alone, might be more easily understood as the result of united action.

The first issue of the official publication of the American Society of Clinical Laboratory Technicians has been received by the JOURNAL. Drs. Philip Hillkowitz, W. E. King, and Kano Ikeda are acting as Associate Editors.

The first Tumor Conference was held at Rochester, Minnesota, during the week of November fifth. The meeting was a success in many ways and sixty-five members and five visitors registered. It is the intention now to have another such conference held in New York City immediately preceding the annual meeting in June.

The following committees have been appointed:

Committee on Local Arrangements

R. A. KILDUFFE, *Chairman*
W. G. EXTON
J. W. GRAY
C. A. PONS
A. YAGUDA

Committee on Necropsies

I. DAVIDSOHN, *Chairman*
C. A. HELLWIG
O. SAPHIR
MARGARET WARWICK

Publication Committee

F. P. MCNAMARA, *Chairman*
F. C. HODGES
M. P. NEAL

Committee on Public Relations

G. L. SCHADT, *Chairman*
M. FERNAN-NUNEZ
G. IVES
B. W. RHAMY

Serologic Conference Committee

A. H. SANFORD, *Chairman*
A. S. GIORDANO
W. M. SIMPSON

Necrology Committee

F. C. PAYNE, *Chairman*
G. B. KRAMER
T. C. TERRELL

Program Committee

A. S. Giordano, *Chairman*
F. J. Heck
S. P. Reimann

Committee on Qualifications

A. H. Sanford, *Chairman*
J. H. Black
A. H. Braden

Publicity Committee

T. B. Magath, *Chairman*
A. S. Giordano
W. M. Simpson

Round Table Committee

F. M. Johns, *Chairman*
J. H. Black
J. J. Moore

Research Committee

R. R. Kracke, *General Chairman*

(1) Hematology Division

R. R. Kracke, *Chairman*
F. J. Heck
N. Rosenthal

(2) Hormonology

Anna M. Young

(3) Investigation of Molds

F. M. Johns, *Chairman*
F. W. Shaw
W. D. Stovall

(4) Serology

B. S. Kline

(5) Slide Exchange

N. Enzer

(6) Tumor Registry

O. A. Brines, *Chairman*
A. L. Amolsch
F. W. Hartman
P. F. Morse
C. I. Owen

Consultants:

E. L. Bishop
A. C. Broders
C. M. Hyland

BOOK REVIEWS

Physiology in Health and Disease. By Carl J. Wiggers. Pp. xxviii + 1184. Philadelphia, Lea & Febiger, 1934. $9.00.

This text, written for medical students, clinicians, and progressive practitioners of medicine, is a distinct departure from the usual text in physiology. The author, from his twenty-five years' experience in teaching, has developed a plan that stresses the application of pure science to physiology and correlates physiologic alterations produced experimentally with aberrant manifestations illustrated in patients. Because there was no single text to cover such subject matter, the author has developed this volume. He has deleted the extensive anatomic and histologic discussions that are in most other texts on physiology. He has given detailed information regarding many phases of the subject which is calculated to make the student see the folly of "cramming" for an examination but he has aided the student in comprehending the subject by dividing the book into numerous chapters which are more or less complete in themselves.

The volume is entirely too large to review completely in a reasonable space, but one may gather some notion as to the subject matter embraced by examining the titles of the major subdivisions which are as follows: The Physiology of Movement, The Physiology of the Peripheral and Central Nervous Systems, The Coördination of Visceral Functions, The Blood and Blood-Forming Organs, Respiration, Heart and Circulation, Physiology of the Alimentary Tract, Water Transport and Excretory Systems, Metabolism and Nutrition, Endocrine Organs, Reproduction.

The entire book is certainly presented from the standpoint of the application of physiology to the practice of medicine and undoubtedly will serve as a practical stimulus to medical students. The author has been very liberal in the citation of references to the literature and at the end of each chapter is appended a list

of monographs and reviews on the subject. It is evident that the book is not intended for the casual reader but for the serious student of physiology.

Green's Manual of Pathology. By H. W. C. Vines. Pp. xii + 928. New York City, Wm. Wood and Co., 1934. $6.50.

An attempt to make this well known English work, in use for seventy-three years, suitable for American students has been made by using a well known American pathologist as a joint author. The general arrangement of the book is along orthodox lines. The work is divided into General Pathology, and Diseases of Special Tissues and Organs. Chapters have been added to this edition which cover avitaminosis, diseases of the ductless glands, diseases of the generative tract, and diseases of the breast. More than 150 new illustrations have been added while more than 100 old pictures have been replaced by photographs. In spite of this revision, the book has still some opportunity for improvement.

A rather large section devoted to bacteria and immunology seems out of place. It is an antiquated discussion of the subject, with no particular reference to pathology. The same is true of the section dealing with parasites. Little or no connection is made between the discussion of the morphology of the parasites, which is very superficial, and the lesions they produce. As examples of startling information, one finds that tapeworms in reality are not worms at all and that trichinosis is "probably conveyed to man in pork" and that trichina succumb "only to a temperature of eighty degrees Centigrade." The author states that the only common trematodes in man are the lung fluke and "Schistosomium haematobium," apparently forgetting Clonorchis and the other species of Schistosoma. In this section, as in that on bacteriology, a mixed terminology is used and several errors are detectable, as, for example, in the life history of the broad tapeworm of man. One finds nine words devoted to blastomycosis with no reference to its pathology, and actinomycosis is said most commonly to affect the region of the cecum and less commonly the mouth which, of course, is exactly the reverse of the facts.

Some of the colored plates could be deleted to advantage, and some, illustrating conditions of the blood, should be radically revised. Pictures of the normal blood and of leukocytosis reveal ratios between leukocytes and erythrocytes all out of proportion to what actually exists; in the discussion of blood groups, one finds no reference to the international classification. Some dogmatic statements such as the fact that sprue is attributable to infection with monilia should certainly be deleted, and it would seem that sporotrichosis would at least deserve mention.

In reference to the chapters dealing with pathology itself, the information contained is pertinent, well written, and for the most part beautifully illustrated. This is particularly true of the chapter dealing with tuberculosis and the section dealing with neoplasms. One gains the distinct impression that if the book were limited strictly to pathology of tissues, and if the material dealing with the subjects of bacteriology and parasitology were deleted, except as they specifically apply to tissue pathology, the text would make a better appeal.

The lack of bibliographic references will not serve as a stimulus to students who should be expected to be more seriously concerned with the important subject of pathology than to be satisfied to learn it from a text.

Clinical Pathology of the Jaws. By Kurt H. Thoma. Pp. xii + 643. Springfield, Charles C. Thomas, 1934. $9.00.

This monograph deals with a highly specialized subject and one which therefore has been somewhat neglected in the past. The scope of the work may be anticipated from the titles of the chapters; they deal with malformations of the head, face, and jaws; atrophy; fractures; infections; endocrine disturbances; nutritional disturbances; general disturbances of uncertain etiology; various cysts, and tumors.

The general structure of the chapters is such that a brief introduction is followed by some general considerations of the disease, and then follows a group of cases, with a brief presentation of history, examination, laboratory findings, diagnosis, and treatment. These cases are beautifully illustrated with 423

figures; photomicrographs, photographs of gross specimens, roentgenograms, and a number of excellent color plates. Particular stress is laid on gross pathology and histopathology, which will make a particular appeal to clinical pathologists. The numerous references at the ends of the chapters make the book suitable for reference work. All in all, it is a thorough treatment of a special group of cases, and of conditions as they occur in a limited region of the body. An appendix contains data on some routine staining methods; the index of cases is useful.

The Patient and the Weather. By William F. Petersen. Pp. xvi + 375, Vol. III. Ann Arbor, Edwards Brothers, Inc., 1934. $5.00.

This is the third volume which deals with the relation of patients to weather. The book contains detailed information about patients who have mental and nervous diseases and cannot be adequately reviewed here.

Textbook of Pathology for Nurses. By Coleman B. Rabin. Pp. 243. Philadelphia, W. B. Saunders Co., 1934. $1.75.

This book was developed from lectures given to nurses in Mount Sinai Hospital, New York City. The subject of pathology is presented in a simple manner, understandable to nurses of average experience and education. It is divided into two general sections, one dealing with general pathology, the other with clinical pathology. The section dealing with clinical pathology chiefly concerns itself with instructing nurses how to collect material for the laboratory and in carrying out some simple laboratory procedures. The book is well illustrated with sixty-one figures.

American Journal of Clinical Pathology

Manuscripts and books for review should be sent to Dr. Thomas B. Magath, Mayo Clinic, Rochester, Minnesota. Manuscripts must be typewritten and all figures and tables should be in such form as to be ready for the printer. The expense for a limited number of cuts can be borne by the Society; expense for cuts in excess of this number will have to be defrayed by the author. The nomenclature for species of bacteria will be that given in Bergey's "Manual of Determinative Bacteriology." Bibliographic references will be limited to the papers actually referred to in the text. Such citations must be arranged in alphabetic sequence and made in the following form: author's name followed by initials, title, journal, volume, inclusive pages, date.

(Examples) Kolmer, J. A.: Toxin production by *Spirochaeta pallida*. Arch. Derm. and Syph., 20: 189–190. 1929.

McFarland, Joseph: A text book upon the pathogenic bacteria and protozoa for students of medicine and physicians. Philadelphia and London: W. B. Saunders Company, 1919, pp. 858.

A table showing cost of reprints, with an order slip, is sent with proof.

Correspondence concerning business matters should be addressed to The Williams & Wilkins Company, Publishers of Scientific Books and Periodicals, Mount Royal and Guilford Avenues, Baltimore, U. S. A.

The AMERICAN JOURNAL OF CLINICAL PATHOLOGY is issued bimonthly appearing in January, March, May, July, September and November. Each volume will consist of approximately 500 pages. Subscription is by the volume only and not by the year. One volume a year is issued at present.

Subscription price: $5.00 per volume in United States, and countries within the postal union; $5.50, countries outside the postal union. The subscriptions of members of the American Society of Clinical Pathologists are included in the membership dues and are handled by the Association's Secretary, Dr. A. S. Giordano, 604 N. Main Street, South Bend, Indiana. All other subscriptions should be sent to the publishers.

Claims for copies lost in the mails must be received within 30 days (90 days, foreign) of the date of issue. Changes of address must be received at least two weeks in advance of issue.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be credited to the next volume, and the renewal invoice therefor adjusted accordingly.

Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, should be sent to the Publishers or to Agents listed below.

AGENTS

For Argentina and Uruguay: Beutelspacher y Cia, Sarmiento 815, Buenos Aires.

For Australia: Angus & Robertson, Limited, 89–95 Castlereagh Street, Sydney.

For Belgium: Henri Lamertin, 58 Rue Coudenberg, Bruxelles.

For the British Empire, except Australia and Canada: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, WC. 2, London, England.

For Canada: Wm. Dawson & Sons, Ltd., 91 Queen Street East, Toronto.

For China: Commercial Press, Ltd., Paoshan Road, Shanghai, China.

For Denmark: H. Hagerup's Boghandel, Gothersgade 30, Kjöbenhavn.

For France. Emile Bougault, 48 Rue des Ecoles, Paris.

For Germany: B. Westermann Co., Inc., Zimmerstrasse 35–41, Berlin SW-68, Germany.

For Holland: Scheltema & Holkema, Rokin 74–76, Amsterdam.

For Japan and Korea: Maruzen Company, Ltd. (Maruzen-Kabushiki-Kaisha), 6 Nihonbashi Tori-Nichome, Tokyo; Fukuoka, Osaka, Kyoto, and Sendai, Japan.

For Spain: Ruiz Hermanos, Plaza de Santa Ana, 13, Madrid.

THE WILLIAMS & WILKINS COMPANY

Publishers of Scientific Books and Periodicals

BALTIMORE, U. S. A.

PUBLISHERS OF: *Medicine, Journal of Urology, Journal of Pharmacology and Experimental Therapeutics, American Journal of Tropical Medicine, Journal of Immunology, Journal of Industrial Hygiene, Quarterly Review of Biology, Journal of Bacteriology, Annals of the Pickett-Thomson Research Laboratory, Chemical Reviews, Soil Science, Social Forces, Personnel Journal, Journal of Comparative Psychology, Mental Measurement Monographs, Studies in Psychology and Psychiatry, Occupational Therapy and Rehabilitation, Child Development, The American Journal of Clinical Pathology, Journal of Physical Chemistry, Philosophy of Science, Medico Legal and Criminological Review.*